GROWTH
Genetics & Hormones
Vol. 11 No. 1 March 1995

Osteochondrodysplasias With Mild Clinical Manifestations: A Guide for Endocrinologists and Others

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Increasingly, individuals with various osteochondrodysplasias are referred for endocrinologic assessment for consideration of growth hormone (GH) therapy. Experimental protocols for such treatment have become more common,[1-3] and thus the involvement of endocrinologists is more complicated than simply recognizing that a child has a chondrodystrophy in order to exclude him or her from further investigation or treatment. Nonetheless, whether hormonal treatment is considered appropriate,[4] rational intervention for genetic counseling and therapy requires accurate diagnosis.

Osteochondrodysplasias are inherited disorders of cartilage and bone, many of which result in short stature secondary to decreased growth of long bones and/or the spine. Hundreds of specific constitutional disorders of bone are recognized.[5] Of these, this review will cover only those disorders with short stature as the primary feature and subtle external features. It is these disorders for which the initial referral may be to a pediatric endocrinologist instead of a geneticist or other specialist with expertise in the diagnosis and/or care of individuals with skeletal dysplasias. Those disorders to be considered here include achondroplasia, hypochondroplasia, pseudoachondroplasia, multiple epiphyseal dysplasia, spondyloepiphyseal dysplasia tarda, and certain mild metaphyseal dysplasias. Clinical characteristics that should lead one to suspect an osteochondrodysplasia and temporal points of referral, defined as when contact with an endocrinologist is most likely, will be pointed out and are compared in Table 1 (page 2). More detailed descriptions of these and other bone dysplasias are found elsewhere.[6-9]

ACHONDROPLASIA

Achondroplasia[10-11] was a general term applied to many forms of short-limbed dwarfism prior to the recognition that vast clinical heterogeneity of the osteochondrodysplasias occurs. It is now recognized that achondroplasia is a single, relatively homogeneous entity. Most often diagnosed in the newborn period, achondroplasia also is frequently diagnosed by prenatal ultrasonographic assessment. It is a useful paradigm with which to compare other, more subtle bone dysplasias.

Diminished linear growth is present from birth.[12] Therefore, diagnostic referral is most likely to occur in the first months of life. Short stature is disproportionate because of shortening of the limbs, particularly the rhizomelic or proximal segments. **Such disproportion and dyssynchronous growth are defining characteristics of most skeletal dysplasias.** In addition, infants and children with achondroplasia have skin redundancy of the rhizomelic segments (Figure 1, page 2), macrocephaly, midfacial hypoplasia (Figure 2, page 3), and a variety of other distinctive features, including hypotonia, which may arise secondary to dyssynchronous growth of the

Table 1
Key Clinical Features of Selected Osteochondrodysplasias

	Achondroplasia	Hypochondro-plasia	Pseudo-achondroplasia	Multiple Epiphyseal Dysplasia	Spondylo-epiphyseal Dysplasia Tarda	Metaphyseal Dysplasias
Short at Birth	Yes	Usually not	No	No	No	Usually not
Onset of Decreased Growth Velocity	Infancy	Early childhood	Early childhood	Late childhood	Preadolescence	Early childhood
Short Limbs	Yes; rhizomelic	Yes; rhizomelic	Yes; later in onset	Mild	No	Subtle to marked
Skin Redundancy	Yes	Variable	No	No	No	No
Macrocephaly	Yes	Sometimes	No	No	No	No
Joint Hypermobility	Yes; particularly hips and knees	Mild or absent	Yes; particularly wrists and hands	No	No	Variable
Upper:Lower Segment Ratio	↑	↑	↑	Normal	↓	Variable
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant (↑ gonadal mosaicism)	Autosomal dominant (usually)	X-linked recessive	Various

foramen magnum and the spinal cord,[13] and joint hypermobility, another common feature of osteochondrodysplasias, which may be helpful in distinguishing such disorders from abnormalities of linear growth not intrinsically affecting cartilage. Although achondroplasia is considered easy to diagnose, severity of manifestations may not be obvious, as is often assumed. Two young children with relatively mild, although unequivocal, manifestations, whose features might not be readily recognized by casual observation, are pictured in Figure 1.

As with all osteochondrodysplasias, specific diagnosis is ultimately dependent upon radiographic assessment. While a complete skeletal survey is needed for definitive diagnosis in this and other bone dysplasias, a limited radiographic assessment will allow for recognition that a bone dysplasia exists. For this purpose, an anteroposterior (AP) view of the hand and wrist, an AP and lateral view of the thoracolumbar spine, an AP view of the pelvis and hips, and an AP view of 1 knee will suffice.

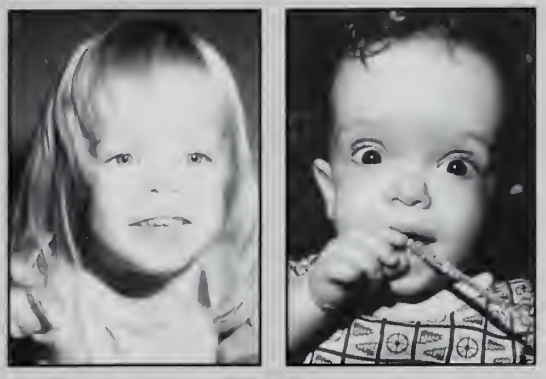
The importance of timely diagnosis is exemplified by the small but real risk of sudden unexpected death or profound neurologic sequelae occurring secondary to cord compression at the craniocervical junction. These risks can be avoided or minimized by appropriate anticipatory evaluation and/or with surgical intervention, which is desirable in a small proportion of babies with achondroplasia.[14-17]

HYPOCHONDROPLASIA

Hypochondroplasia[18] is another rhizomelic dwarfing disorder. Perhaps more than with any other osteochondrodysplasia, individuals with hypochondroplasia may be misdiagnosed as having familial short

Figure 1
Achondroplasia: Two Children With Relatively Mild Phenotypic Features

Figure 2
Achondroplasia: Macrocephaly, Craniofacial Disproportion, and Midface Hypoplasia Are Always Present But Quite Variable in Severity



stature, since the short stature often is relatively mild and body disproportion frequently is less obvious than in achondroplasia. Macrocephaly is not uniformly present and joint characteristics are not prominent (Figure 3).

Individuals with hypochondroplasia often have birth weights and lengths within the normal range, in contrast to achondroplastic infants. This and the subtlety of their disproportionate growth mean that the growth disorder is more likely to be recognized at 2 to 4 years of age. Recognition is further compromised by the considerable variability of growth abnormalities, with adult stature ranging from as little as 125 cm to as much as 160 cm. Indeed, in mildly affected individuals, clinical and radiographic features may not be clearly distinguished from variations of normal.

PSEUDOACHONDROPLASIA

Like achondroplasia and hypochondroplasia, pseudoachondroplasia (or spondyloepiphyseal dysplasia of the pseudoachondroplastic type) is a single gene, dominant condition.[19-20] It is unusual among the dwarfing osteochondrodysplasias because clinical and radiographic manifestations may be delayed and often are not evident until after the first year of life. Early growth remains within the normal range and disproportionate growth is not immediately recognizable. Indeed, **typical growth in an individual with pseudoachondroplasia[21] mimics the growth failure** that may be seen, for example, **in postnatal endocrinologic disturbances** rather than the growth characteristics usually associated with intrinsic abnormalities of bone. It also serves to emphasize that **abnormal craniofacial characteristics are unreliable as indicators of the presence of an osteochondrodysplasia.** Indeed,

Figure 3
Hypochondroplasia: Rhizomelic, Disproportionate Shortening May Not Be Evident Without Complete Clinical Assessment

people with pseudoachondroplasia have normal facial features and no craniofacial disproportion (Figure 4, page 4).

Nonetheless, certain clues should lead one to suspect this diagnosis early in life, including unusual gait (secondary to hip involvement); malalignment of the legs; and joint hypermobility, particularly of the hands and the wrists. Such features reflect general cartilaginous involvement. In a variety of conditions, clinical features of cartilaginous abnormality are important clues to the presence of an osteochondrodysplasia.

MULTIPLE EPIPHYSEAL DYSPLASIA

Individuals with multiple epiphyseal dysplasia have often only very subtle growth disturbances.[22,23] Indeed, abnormalities of growth may remain unrecognized until late childhood or early adolescence. Body habitus is virtually normal and the face is unaffected. Most individuals will present because of either slowing growth rate or progressive joint involvement with pain, stiffness, and/or abnormality of gait. This entity should be considered when constitutional delay of growth is suspected. The epiphyses are the parts of bone that are affected, and X-ray films of these often raise the index of suspicion. Epiphyseal irregularity may be subtle and vary from site to site. The primary radiographic manifestation may be delayed epiphyseal development.

Figure 4

Pseudoachondroplasia: These 3 Girls Exemplify the Completely Nondysmorphic Facial Features of This Osteochondrodysplasia

This delay can confuse the diagnosis, particularly if only a hand and wrist film is obtained, and underscores the point that **delayed bone age is not necessarily the result of endocrinologic processes but can equally be secondary to an osteochondrodysplasia.**

SPONDYLOEPIPHYSEAL DYSPLASIA TARDA

Spondyloepiphyseal dysplasia (SED) tarda, unlike SED congenita, which is a severe disorder easily recognized in the newborn period, is usually not recognized until the immediate preadolescent period.[24] Even at that age, hip and back symptoms more often than small stature result in medical referral; however, the modest apparent slowing of growth may precipitate referral to an endocrinologist. The growth disturbance results almost exclusively from vertebral involvement, which causes a short trunk. **Additional clinical measurements, such as span:height and upper:lower segment ratios, are helpful** in raising the index of suspicion that a short-statured individual may have such a short trunk osteochondrodysplasia. Similar measurements are seen in boys and girls with congenital anomalies of the vertebrae. Since SED tarda is most often an X-linked recessive disorder, only males are affected by the classic form of this disorder.

In Future Issues

The Neuroendocrine Landmarks of Puberty
by Jean-Pierre Bourguignon, MD, PhD

Imaging in Diagnosing Hypopituitarism
by Raphael Rappaport, MD

METAPHYSEAL DYSPLASIAS

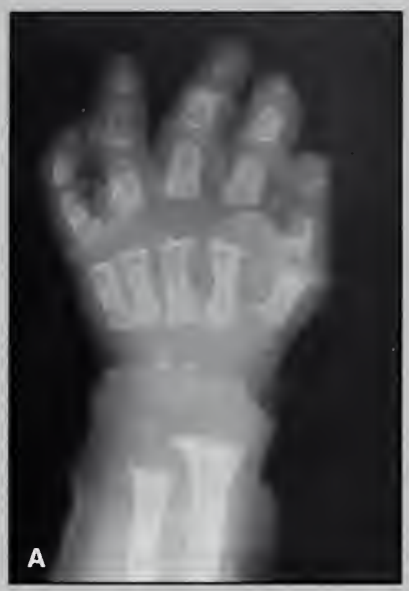
A variety of osteochondrodysplasias affect principally the metaphyses of the long bones. They range from quite subtle anomalies of growth, eg, Schmid type of metaphyseal dysplasia, to potentially lethal conditions, eg, cartilage-hair hypoplasia. The Schmid type[25] is the most common and best delineated form of metaphyseal dysplasia. Usually, individuals with this disorder are not recognized as being in any way abnormal until around 2 years of age. Often leg bowing and/or joint prominence are the first signs, rather than slowing growth. Nonetheless, slowing of growth ultimately results in adult heights ranging from about 130 cm to 160 cm.

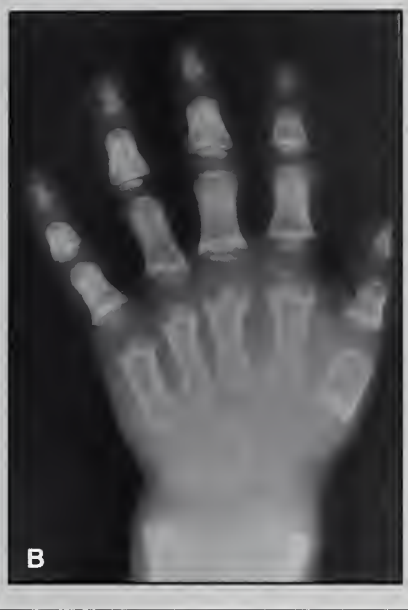
SUMMARY AND RECOMMENDATIONS

A series of concepts and caveats that might be helpful to clinicians have been generated (Table 1). Suspicion that an osteochondrodysplasia may be present should be raised when:

- Disproportionate and dyssynchronous growth are present, since these are defining characteristics of most skeletal dysplasias. Such disproportion may result in a short trunk as in SED tarda, in primarily short limbs as in hypochondroplasia, or in disproportionate limb segments as in achondroplasia. Additional segment measurements and additional calculations, such as span and sitting height measurements and calculations of the upper:lower segment ratio, are important in clinically differentiating the milder osteochondrodysplasias. Growth patterns themselves may *not* allow differentiation of endocrine from osteochondrodysplastic causes since some bone dysplasias show normal or near-normal growth early in life and subsequent evidence for growth failure, eg, pseudoachondroplasia, SED tarda, metaphyseal dysplasias.

Figure 5
Hand and Wrist X-ray Films





A) 15-month-old achondroplastic female. Note minimal metacarpal deformity, short proximal and middle phalanges, and brachydactyly.

B) 48-month-old pseudoachondroplastic female. Note marked delay in carpal development, shortening of all long bones, and widened and unusually configured metaphyses.

- Craniofacial disproportion is recognized, as occurs in achondroplasia. Nonetheless, abnormal craniofacial characteristics are not uniformly present in osteochondrodysplasias, eg, pseudoachondroplasia and metaphyseal dysplasias.
- Features suggesting generalized cartilaginous abnormality are found. Often this will result in such joint characteristics as ligamentous laxity, as in pseudoachondroplasia; joint hypermobility, as in achondroplasia; joint pain, as in multiple epiphyseal dysplasia; or joint prominence, as in metaphyseal dysplasias.
- Specific radiographic features are discovered. Specific diagnoses of all osteochondrodysplasias ultimately rest almost exclusively with radiographic identification. Nonetheless, as with clinical features, radiographic manifestations may not be evident from birth, eg, pseudoachondroplasia. Hand and wrist films usually are obtained in children referred for endocrinologic assessment of small stature. Since they often will demonstrate specific features of a bone growth disorder, these should always be reviewed not only for establishing bone age but also for discerning subtle and not so subtle features of osteochondrodysplasias (Figure 5). It should be stressed that when radiographic assessment is limited to the hand and wrist, some osteochondrodysplasias may show only delayed bone age, eg, multiple epiphyseal dysplasia. A delay in skeletal maturation should not be misinterpreted as unequivocally reflecting an endocrinologic process. X-ray films of other joint sites may reveal that chondrodystrophy is responsible for the short stature. While a skeletal survey of all bones and joints is needed for definitive diagnosis of specific osteochondrodysplasias, the endocrinologist's role might be viewed as more limited: determining that some osteochondrodysplasia is present in order to initiate appropriate referral. For this purpose, an AP view of the hand and wrist, an AP and lateral view of the thoracolumbar spine, an AP view of the pelvis and hips, and an AP view of 1 knee will suffice.

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Letter From the Editor

To Our Readers:

Welcome to the **10th birthday of *GROWTH, Genetics, & Hormones*** *(GGH)*! Now that 10 years of publication have been completed, a historical accounting of our goals and accomplishments is in order. On this occasion, an expression of sincere gratitude and appreciation from us, the Editorial Board, and from you, the 12,000-plus readers, is extended to Genentech, Inc., which has been particularly insightful and/or responsive to the need for this newsletter. *GGH* has been generously supported by Genentech, Inc. through an educational grant, and published by an Editorial Board that operates totally independently from Genentech.

Historically, *GGH* was created with the vision of providing an objective and unfettered opportunity for teachers and scientists in the areas of growth, endocrinology, genetics, and nutrition. We believe that those goals continue to be met. Forty-one issues of *GGH* have been published, comprised of 91 lead articles and 313 abstracts of journal articles — each with at least 1 editorial comment; 20 reviews of important medical conferences and/or meetings; a glossary of genetic terms; a tabulation from the genome project of the chromosomal sites of most of the genes related to growth and hormones; and a significant supplement about the ethics of growth hormone use. Over 12,000 physicians, nurses, and medical libraries receive *GGH* every quarter.

Verification that the goals are being met and that the readers of *GGH* hold it in high esteem was obtained recently through replies to a reader survey, distributed with the September 1994 issue. The response to the survey overwhelmingly confirmed the goals continue to be met and the readers very much appreciate that which was created by the donor and the recipient of the educational grant. As of the first of January, 2,210 responses were received from the 12,000-plus recipients of *GGH*. By November 9, within 6 weeks of receipt of the survey, 1,290 readers had responded. Of these, more than 800 replies came from endocrinologists and geneticists, and the remainder were from nephrologists, practicing and academic pediatricians, nurses, and others. Eighty-four percent of the 1,290 placed *GGH* as a significant priority on their reading list each quarter, and 50% of these rated it as a *very high* or *high priority*. As of November 9, 64% of respondents stated that they publish, and 20% of these cite *GGH* in their references — a phenomenal reflection of the respect that *GGH* has among contributors to the literature. The current Editorial Board members – Dr. William Clarke, Jr; Dr. Judith Hall; Dr. William Horton; Dr. Fima Lifshitz; Dr. Allen Root; and myself – express our thanks most sincerely to you our readers for having replied promptly. Again we thank Genentech Corporation for the opportunity for *GGH* to have met, and to continue to meet, the goals established 10 years ago. The Editorial Board also recognizes and thanks the distinguished previous members of the Board who contributed in a laudatory manner. These include Dr. Jurgen Bierich of Germany (now deceased); Dr. Alan Rogol and Dr. David Rimoin of the United States; Dr. James Tanner of England; and Dr. Jean-Claude Job of France.

We conclude by noting that *GGH* is requested and read by individuals of many disciplines in many countries (more than 500 readers in Europe), and is made available in many medical school and hospital libraries in the western hemisphere.

HAPPY BIRTHDAY, *GGH* !!!

Thank you,

Robert M. Blizzard, MD
Editor-in-Chief
For the Editorial Board

Abstracts From the Literature

Catch-up Growth After Glucocorticoid Excess: A Mechanism Intrinsic to the Growth Plate

In an attempt to better understand catch-up growth and determine whether the mechanism governing such growth resides in the central nervous system or in the growth plate, the authors devised a series of novel experiments using rabbits. Stainless-steel needles (27 gauge) were inserted through the proximal tibial growth plate and attached to an osmotic pump, which administered dexamethasone continuously for 4 weeks. Similar incisions and needle placements were made on the contralateral tibia. Three metal pins were placed: 1 in the bony metaphysis 2 to 3 mm distal to the growth plate; 1 that was 5 mm distal to the first pin; and 1 that was 5 mm proximal to the first pin. Radiographs of the tibiae were examined under a dissecting microscope and the distances between the proximal and middle pin, the middle pin and the distal pin, and the distal pin and the distal tibial epiphysis were measured. All measurements were done in duplicate. The distance between the middle and distal pins did not span a growth plate and was therefore expected to remain constant. Measurement of

this distance was used as a determinant of the variability of measurement.

After 4 weeks of dexamethasone administration, the treated proximal tibia grew 37% ± 8% less than the contralateral control proximal tibia. Growth inhibition resolved once dexamethasone was stopped, and the growth rates on the 2 sides were equalized by week 6 (2 weeks after the end of the infusion). The observed growth velocity in the treated growth plate surpassed that of the contralateral growth plate and corrected 54% ± 13% of the growth deficit. The distal tibial growth plates did not change during the period of catch-up growth, ie, there was no significant difference in cumulative growth at the distal growth plate. In addition, femoral length showed no significant discrepancies at the end of the experiment, suggesting that the increased growth was observed only in the growth plate in which growth inhibition had occurred.

Baron J, et al. *Endocrinology* 1994;135:1367-1371.

Editor's comment: *Although it is not usually the policy of* GGH *to abstract animal studies, this particular study should be of sufficient interest to be included here. These data suggest that catch-up growth is intrinsic to the growth plate and not the result of a systemic hormonal mechanism. The authors also have demonstrated that catch-up growth may not be 100% complete. They suggest that this may be due to the number of stem cells or chondrocytes present in the affected growth plate.*

This study is important for what it tells us about the mechanisms of growth retardation and catch-up growth. In humans, as in the animal model, catch-up growth is presumably intrinsic to the growth plate and the removal of the growth inhibitor rather than a response to changes in hormonal secretion. It would be interesting to see similar studies performed in models of other disorders associated with decreased growth velocity and subsequent catch-up growth.

William L. Clarke, MD

Sex, *SOX*, and the Skeleton

It is well established that the *SRY* (sex-determining region Y) gene is critical for testicular development in mammals. Since its protein product contains a DNA-binding motif that is found in many transcription factors, it is assumed that it functions to regulate the expression of relevant genes. Recently, a family of structurally related genes, the *SRY*-related genes, so-called *SOX* genes, has been identified. In the paper by Foster and colleagues, mutations of the *SOX9* gene were discovered to cause campomelic dysplasia (CD) with sex reversal. This disorder has always been an enigma because it was hard to understand the connection between abnormal bone growth and sex reversal.

Previous reports of chromosomal rearrangements in CD with sex reversal had localized the gene(s) responsible to the long arm of chromosome 17, specifically 17q24.1-q25.1. Foster et al carried out high-resolution mapping of this region to position a translocation breakpoint in one patient with this disorder close to the *SOX9* locus, which had been previously mapped. They next characterized the structure of the *SOX9* gene, showing that the predicted polypeptide would be 509 amino acids in length and contain the DNA-binding motif. Fluorescence in situ hybridization (FISH) confirmed its localization to chromosome 17q24; and northern blot analysis demonstrated the presence of mRNA transcripts in adult testes as well as in adult heart and fetal brain.

Next, they utilized single-strand confirmation polymorphism (SSCP) analysis to find 6 mutations in DNA from 9 patients with CD. Three would be expected to abolish gene function by shifting the reading frame of the mRNA transcripts so that translation would be prematurely terminated. In 2 cases, about one third of the protein would be lost, and in 1 case about 60% would be lost. The missing part contains the putative activation domain of the protein. Another mutation predicted that splicing of *SOX9* transcripts would be altered, and 2 others predicted that amino acids would be substituted in the protein. The mutations were not present in parental DNA, indicating that they arose de novo. Also, they were not found in surveys of normal individuals. Importantly, the mutations were heterozygous, denoting that the disorder behaves in an autosomal dominant manner.

The authors speculated about how mutations in the *SOX9* gene might cause CD with sex reversal. Since the mutations were predicted to destroy the function of the gene product and since the patients were found to have both a mutant and a normal *SOX9* allele, they proposed that the mutations operate through a loss of function mechanism, ie, haploinsufficiency, rather than through gain of function or dominant negative mechanisms. They noted that dosage sensitivity is a feature of many regulatory genes and has been reported for several sex determination systems. They further pointed out that the sex-determining function of *SRY* is believed to be expressed in pre-Sertoli cells in the developing gonadal ridge. They raised the possibility that interactions between *SRY* and *SOX9* gene products may be necessary for normal testicular development, perhaps by influencing the behavior of mesenchymal cells in the ridge.

Wright and coworkers shed light on the situation by demonstrating that the mouse *SOX9* gene is expressed abundantly in regions of the developing skeleton just before cartilage forms. The normal developmental sequence is that mesenchymal cells in areas destined to become skeleton "condense" shortly after which they begin to generate cartilaginous molecules that assemble into templates of future bones. The observations of Wright et al suggest that *SOX9* expression activates the chondrocytic developmental program and, as such, acts as a master gene for chondrogenesis, thereby performing a role somewhat analogous to MyoD in myogenesis.

The authors also provided strong linkage evidence that a mouse skeletal mutant called Tailshort (Ts) maps to the *SOX9* locus and may be the mouse equivalent of CD in humans. Interestingly, Ts mice do not exhibit abnormalities of sexual development.

Foster JW, et al. *Nature* 1994;372:525-530.
Wright E, et al. *Nat Genet* 1995;9:15-20.

Editor's comment*: Envisioning the relationship between defective testicular and skeletal development in CD has always been difficult. These papers provide considerable insight into this matter. Given that its expression coincides spatially and temporally with early skeletal development, one can postulate*

many ways in which SOX9 *mutations could disrupt skeletogenesis. Indeed, it is surprising that the clinical features are not more severe than are typically found. The connection between* SOX9 *mutations and defective testicular development is less obvious. However, the similar structure of* SOX9 *and* SRY *provides a good basis for speculation. The suggestion of Foster et al that the transcription factor products of the 2 genes may need to interact to carry out their normal function during testicular differentiation seems tenable and is supported by another recent paper by Wagner et al, which demonstrated* SOX9 *expression in the fetal testes. Although unproven, the idea that* SOX9 *is a master gene for chondrocytic differentiation is very enticing, since many researchers have been looking very hard for such a gene with little success. It will be very interesting to see how this story plays out.*

William A. Horton, MD

Wagner T, et al. *Cell* 1994;79:1111-1120.

The Genes for Crouzon Craniofacial Dysostosis and Pfeiffer Syndrome Are Fibroblast Growth Factor Receptor Genes

Crouzon craniofacial dysostosis (CFD) is an autosomal dominant inherited disorder characterized by premature closure of the cranial sutures (craniosynostosis), shallow orbits, and hypoplastic maxillae. The incidence of Crouzon syndrome has been estimated to be 1/25,000. It has been associated with advanced paternal age, and at least 50% of reported cases are thought to be due to de novo mutations.

A recent paper by Preston et al studied 2 very large kindreds affected with CFD in which they successfully mapped the gene for CFD to the long arm of chromosome 10. They used a candidate locus approach because no consistent cytogenetic abnormalities have been found in CFD. They first tried mapping the CFD gene to chromosome 7p, because of the similarities found between CFD and Greig cephalopolysyndactyly, which has been localized to 7p13 (Brueton et al [1988a]; Brueton et al [1992]) and Saethre-Chotzen syndrome, which has also been mapped to the 7p21 region (Brueton et al [1988b). Their initial mapping results, however, did not support linkage to chromosome 7p. Based on the evidence that mutations in developmental control genes in mice cause abnormal morphogenic phenotypes similar to CFD, they continued their search in regions known to contain developmental regulatory genes (*HOX*, *PAX*, *POU*, and zinc finger genes). Their results showed that both families had linkage to chromosome 10q25-q26.

Preston et al's mapping work was rapidly followed by a paper by Reardon et al in which they presented evidence that mutations in the fibroblast growth factor receptor 2 gene (*FGFR2*) cause Crouzon syndrome. Fibroblast growth factor (*FGF*) receptors act by binding activating-specific cell-surface receptors. *FGFR2* has been shown to map to human chromosome 10q25.3-26 and has 2 alternative gene products, KGFR (keratinocyte growth factor receptor) and BEK (bacterially expressed kinase). These 2 gene products have different patterns of expression in murine embryogenesis, with the BEK gene transcripts concentrated in the frontal bones, maxillae, mandibulae, and ossicles in the middle ear.

This association and the recent report of linkage of CFD to 10q25-q26 led Reardon et al to study 20 CFD cases and 89 unaffected controls by amplifying the coding sequence and splice junctions of *FGFR2*. The product was analyzed by single-stranded conformational polymorphism (SSCP) analysis. They found alterations of SSCP migration proteins in 9 of 20 CFD cases and a variety of shift bands in the other patients, indicating a heterogenous range of mutations. Further study of these 9 cases showed that 3 had a G→A transition at nucleotide 1037 resulting in a Cys342Tyr substitution within the third immunoglobulin domain. The other patients had a variety of different transitions in nucleotides 1036, 1030, 1073, and 1044. No SSCP variations were found in unaffected individuals.

Jabs et al analyzed the same region of the *FGFR2* gene in additional families with CFD and in a family with the related syndrome, the Jackson-Weiss syndrome. The latter syndrome has many of the cranial features of the former but differs in that they are more variable and also that patients have foot deformities, including broad great toes with medial deviation and tarsal-metatarsal coalescence, and occaionally hand malformations. In the CFD patients, they confirmed 2 of the previously described heterozygous mutations and identified 2 others, both of which would be predicted to introduce additional cysteines into the third Ig domain of the protein. In the Jackson-Weiss family they found a mutation predicted to substitute a glycine for an alanine at residue 344, only 2 amino acids from the cysteine 342 mentioned above.

Given these observations and the fact that they had recently mapped a similar autosomal dominant syndrome, the Pfeiffer syndrome, to chromosome 8p11.2-p12, where another FGFR gene, *FGFR1*, resides, Muenke and coworkers did the obvious. They analyzed the structure of *FGFR1* in a large family with Pfeiffer syndrome. This syndrome is characterized by premature fusion of several sutures of the skull, broad thumbs and great toes, short fingers and toes, and variable degrees of syndactyly. They began their analysis by PCR amplifying exons 3 through 7 of the *FGRF1* gene. These exons code for the second and third Ig domains of the receptor. The products were analyzed by SSCP. When an anomaly was detected in a fragment from exon 5 in affected but not in unaffected family members, they sequenced the fragment. A heterozygous single base pair change predicting a proline to arginine substitution at amino acid residue 252 was found. The same heterozygous mutation was subsequently detected in affected members from 4 other Pfeiffer syndrome families. Proline 252 is a highly conserved amino acid that is located between the second and third Ig domains. The authors acknowledge that the specific way in which the mutation causes the clinical phenotype is not known.

Preston RA, et al. *Nat Genet* 1994;7:149-153.
Brueton J, et al. *Am J Med Genet* 1988;31:799-804.
Brueton LA, et al. *J Med Genet* 1992;29:681-685.
Reardon W, et al. *Nat Genet* 1994;8:98-103.
Jabs EW, et al. *Nat Genet* 1994;8:275-279.
Muenke M, et al. *Nat Genet* 1994;8:269-274.

***Editor's comment**: It is clear that growth factor receptors play an important role in embryologic development. The previous report of mutations in* FGFR3 *as a cause for achondroplasia and this Crouzon syndrome report suggest cartilage growth is influenced by certain* FGFs *and their receptors. Further studies will be helpful in establishing the roles of the various* FGFRs *and their differential expression in different tissues and body areas. It seems likely these expression patterns will be time-in-development specific as well.*

Readers may wish to review an abstract in GGH *Vol. 10, No. 4 entitled "Snaring the Achondroplasia and Hypochondroplasia Gene." The discussion is related because in these entities the* FGF3 *and* FGFR3 *are involved.*

Judith G. Hall, MD

***2nd Editor's comment**: These papers clearly establish an important link between* FGF *signaling and skeletal development both in the skull and in the distal limbs. When achondroplasia is taken into account, this link is expanded to include development of the spine and proximal limbs as well. On the basis of the disease phenotypes, it is tempting to speculate about how mutations of these genes operate and about the roles that the receptor proteins play during normal skeletal development.*

However, one must be very cautious considering the extreme complexity of FGF *signaling as its story unfolds. For instance, consider that there are at least 9* FGF *ligand and at least 4* FGF *receptor genes. Alternative splicing is known to generate different forms of the receptor proteins. Although both ligands and receptors are thought to dimerize in order for signals to be transmitted, it is not known which ligands bind to which receptors. Moreover, it is suspected that heterodimers may form between different ligands and between different receptors. The downstream signaling pathways are not well defined. To make matters worse, extracellular matrix constituents, such as heparin sulfate, appear to influence diffusion of ligands to target cells and binding of ligands to receptors. Thus, the number and types of signals transmitted by* FGFs *are potentially extremely large and diverse, as are the ways in which* FGF *signaling might be disturbed by mutations.*

Also keep in mind that virtually all of the work on the receptor mutations to date has been carried out on DNA, ie, the amino acid substitutions, and functional sequelae are predicted rather than observed. There may still be surprises as the venture extends to the protein level. Despite these cautions, the tracing of 4 human genetic disorders of skeletal development to 3 receptor genes in such a short period is a very significant accomplishment.

Finally, it is intriguing that, as with achondroplasia mutations in the FGFR3 *gene, mutations of the* FGFR1 *and* FGFR2 *genes tend to cluster at particular sites. As the biology of* FGF *signaling becomes better understood, these observations should provide valuable clues to elucidating the molecular pathogenesis of these disorders.*

William A. Horton, MD

Long-Term GH Therapy in GHD and Non-GHD Boys: Effect on Bone Age and Pubertal Maturation

Zadik and colleagues report the effects of 4 years of therapy with recombinant human growth hormone (rhGH) (0.1 mg/kg 3 times/wk) on the growth of full-term, short, slowly growing males with substantial delay in skeletal maturation (>2 standard deviations [SD] for age) and subnormal 24-hour mean integrated serum GH concentrations (<3.2 ng/mL [double antibody polyclonal radioimmunoassay]). These children were subdivided into those with classic GH deficiency (peak GH secretory response to 2 provocative stimuli <10 ng/mL, n=40) and those whose peak GH responses were >10 ng/mL and were said to have GH neurosecretory dysfunction (n=43). Both groups of children grew more rapidly while receiving rhGH than in the pretreatment period. However, after completing 4 years of treatment, those with classic GH deficiency had a somewhat greater cumulative gain in height than did those with GH neurosecretory dysfunction (+1.6 vs +1.2 SD score), and the predicted adult height increased to a greater extent in the former group (+9.3 vs +5.4 cm).

Loche and coworkers treated 15 short, slowly growing, otherwise normal children (10 males) with delayed skeletal maturation and normal spontaneous and stimulated GH secretion with rhGH. Doses of 0.19 or 0.38 mg/kg/wk were administered in 4 to 7 weekly subcutaneous injections until final height (growth rate <2 cm/y and/or skeletal epiphyseal fusion) was achieved after 5 or more years of therapy. Although children receiving the larger dose of rhGH initially grew more rapidly, both groups of subjects ultimately achieved similar increments in height during therapy. Final heights did not differ significantly from pretreatment predicted adult heights, or from mean target heights (calculated from midparental heights).

Zadik Z, et al. *J Pediatr* 1994;125:189-195.
Loche S, et al. *J Pediatr* 1994;125:196-200.

***Editor's comment:** The report of Loche et al demonstrates that rhGH at the 2 dosages tested did not increase final height of normal short children beyond their predicted or target heights. However, the therapeutic programs were not uniform, and whether larger doses or more frequent administration of rhGH will increase final height is as yet unresolved. It is likely that any such effects will be much more subtle and difficult to demonstrate than the obvious growth-promoting effect of rhGH in children with the somatic and radiographic characteristics of the truly GH-deficient patient. These investigators also demonstrated that growth rate often declined after 5 years of rhGH*

administration. Thus, the report of Zadik et al, in which 4 years of observations are recorded, must still be considered preliminary until this group reports their final height data.

The diagnosis of permanent GH deficiency, particularly when it is an isolated defect, remains difficult. Adan et al[1] reevaluated children in whom the diagnosis of hyposomatotropism had been established and treatment with rhGH administered. They observed that compared with children with transient deficiency of GH (those in whom GH secretion at an older age was normal), patients with permanent GH deficiency were much more likely to have onset of growth failure before 5 years of age; neonatal hypoglycemia; micropenis (males); radiographic evidence of interruption of the pituitary stalk; lower plasma concentrations of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor-binding protein 3 (IGFBP-3) but not stimulated GH secretion at the point of diagnosis; and often several pituitary hormone deficiencies. Cohen and Berg[2] recently noted that 29/34 patients with suspected isolated deficiency of GH had normal GH secretion and/or IGF-1 concentrations when retested at an older age. Since the state of thyroid function may fluctuate in patients with autoimmune thyroid disease, depending on the biology of the autoantibody generated at any one point in the disease, the concept of a transient deficiency of hGH secretion that requires therapy with GH and then remits spontaneously is not necessarily unique. However, there are no data supporting an autoimmune mechanism in the latter disorder, nor are there long-term follow-up reports to document the natural history of this phenomenon in adulthood. Currently, this writer has difficulty with the diagnosis of transient GH deficiency and has attributed most such diagnoses to our inability to identify the truly GH-deficient subject in the absence of findings listed by Adan et al. Marin et al[3] point out just how little GH may be secreted in response to provocative stimulation in children of normal stature, particularly if they are prepubertal. Even with sex hormone priming, the data of these authors indicate that a peak GH concentration of 7 ng/mL (double antibody polyclonal radioimmunoassay) is normal. Thus, the selection of a minimum normal GH value of 10 ng/mL for the diagnosis of GH deficiency is quite arbitrary and not substantiated.

Allen W. Root, MD

1. Adan L, et al. *J Clin Endocrinol Metab* 1994;78:353-358.
2. Cohen AJ, Berg L. Proceedings of the Eighth Annual Investigators Meeting of the National Cooperative Growth Study; October 27-29, 1994; Orlando, Fla.
3. Marin G, et al. *J Clin Endocrinol Metab* 1994;79:537-541.

Longitudinal Analysis of Somatic Development in Paediatric Patients With IDDM, Part 1: Genetic Influences on Height and Weight

Holl et al evaluated height and weight in 389 insulin-dependent diabetes mellitus (IDDM) patients (188 males, 201 females) between the years of 1980 and 1992. All were treated with 2 to 4 daily injections of regular and NPH insulin. All medical care was provided by the same group of health-care professionals. Height was measured with a Harpenden stadiometer until 1989, and then with an electronic stadiometer with automatic recalibration. Bone ages were determined according to the method of Gruelich and Pyle. Families were included only if both parents were nondiabetic and available for measurements. Complete data were available for 177 pairs of parents and 186 unaffected siblings. Height, weight, and body mass index (BMI) standard deviation (SD) scores were calculated yearly. Nonparametric tests were applied.

At the onset of IDDM, the patients were significantly taller compared with normative data (SD score +0.37, $P<0.001$). During the course of the disease, the median Z score for height progressively decreased. But after 10 years, the height decrement was reversed and scores returned to above zero. Even during the first year of diabetes, the children were heavier than the normative sample, (BMI Z score +0.26, $P<0.001$). The weight Z score increased while the height Z score decreased.

Seventy-six patients, assumed to be at adult height (chronologic age, 18 years) had a mean height SD score of +0.30, nearly identical to that of their siblings (+0.22). The Z score for weight at age 18 was +1.06 and for BMI was +1.23, with no significant differences between boys and girls. Both midparental height and midparental weight were significantly related to the respective SD scores for their diabetic children ($r = +0.43$, $P<0.0001$ for height, and $r = +0.23$, $P<0.002$ for weight). At the onset of diabetes, bone ages were not retarded, but they were progressively delayed during the course of diabetes.

The authors conclude that age- and sex-standardized height in diabetic children is not significantly different from the respective measurements in their unaffected siblings and that adult height is not compromised in diabetic individuals.

Holl R, et al. *Diabetologia* 1994;37:925-929.

Part 2: Final Height Attainment in Girls and Boys With Insulin-Dependent Diabetes Mellitus

d'Annunzio et al studied 37 Italian insulin-dependent diabetes mellitis (IDDM) patients (15 males, 22 females) who, at last evaluation, had a mean age of 20.6 ± 3.3 years and an average disease duration of 11.8 ± 3.7 years. Patients were divided into 2 groups on the basis of the presence of pubertal development at diagnosis (20 patients prepubertal, 17 pubertal). All subjects were treated with 2 or more daily injections of a mixture of short- and long-acting insulin. Height at diagnosis and final height were assessed with a Harpenden stadiometer and height was converted to standard deviation (SD) scores. Bone age at diagnosis was determined by the method of Gruelich and Pyle. Predicted adult height was determined by the Bailey and Pinneau method. Target genetic height was assumed to be the mean parental height plus 6.5 cm for males, and the mean parental height less 6.5 cm for females.

Height at diagnosis was variable for boys and girls. It was above the 50th percentile in 10 of 22 females, at the 50th percentile in 6, and between the 3rd and 50th in another 6. Final height in girls was above the 50th percentile in 9, at the 50th percentile in 6, and between the 3rd and 50th in 7. Final height in both males and females was higher than their target genetic height, although not significantly. No difference was observed in final height between patients diagnosed in the prepubertal or pubertal stages. In boys, height at diagnosis was above the 50th percentile in 8, at the 50th percentile in 4, and between the 25th and 50th in 3. Final height was above the 50th percentile

in 7, and between the 10th and 50th in 8. No correlation was observed between final height and glycosylated hemoglobin concentrations, early microangiopathic complications, or thyroiditis.

The authors conclude that there was no growth retardation in their patients, that final height exceeded target genetic height, and that diabetes by itself did not impair final height.

d'Annunzio G, et al. *Diabetes Res Clin Pract* 1994;24:187-193.

***Editor's comment**: These 2 papers from Germany and Italy present similar findings. The data are both welcome and reassuring. Although the literature is replete with descriptions of height at diagnosis of children with diabetes, and examples of poor growth associated with extremely poor glucose control, there have been few data regarding final height in these children. The findings, however, are probably not surprising to those who care for young adults with IDDM, for short stature is not a term frequently used to characterize the adult who has had IDDM as a child.*

What both of these papers fail to clarify is how one is to interpret the array of data currently published with regard to growth parameters in the diabetic child, ie, growth velocity, integrated growth hormone concentration, pulse amplitudes and frequencies, insulin-like growth factor (IGF)-1, IGF-binding protein 3, growth hormone-binding protein, and IGF-binding protein 1. What is the clinical and pathophysiologic significance of these data if the majority of diabetic children reach or exceed their predicted final adult height? Perhaps this question is best posed to the researchers currently publishing these data. Are their patients destined to have short stature, or like the patients of Holl et al, will they eventually regain their growth potential despite early decreased linear growth velocity? Long-term studies of the patients reported in previous studies are very much needed.

William L. Clarke, MD

Preliminary Localization of a Gene for Autosomal Dominant Hypoparathyroidism to Chromosome 3q13

In a kindred in which 7/15 members over 3 generations had mild, generally asymptomatic hypocalcemia associated with hyperphosphatemia and low or inappropriately normal concentrations of parathyroid hormone, linkage to chromosome 3q13 was established. This region of chromosome 3q is near that for the parathyroid cell Ca^{++}-sensing membrane receptor mapped to chromosome 3q2 (Brown et al and Pollak et al). The investigators suggest that a mechanism opposite to that identified in patients with familial hypocalciuric hypercalcemia, in which the sensitivity of the receptor for Ca^{++} is decreased or downregulated, is operative in the present family. This would mean that receptor sensitivity is upregulated and, therefore, lower concentrations of Ca^{++} are required to depress the secretion of parathyroid hormone.

Finegold DN, et al. *Pediatr Res* 1994;36:414-417.
Brown EM, et al. *Nature* 1993;366:575-580.
Pollak MR, et al. *Cell* 1993;75:1297-1303.

***Editor's comment:** This report illustrates yet another possible mechanism for familial hypoparathyroidism, in addition to abnormalities within the gene for parathyroid hormone itself (chromosome 11p15), which is transmitted as an autosomal recessive characteristic, and embryonic dysgenesis of the parathyroid gland, which is inherited as an X-linked recessive trait. One awaits analysis of the gene for the Ca^{++}-sensing receptor in this family and its expressed characteristics.*

Allen W. Root, MD

Growth Hormone Releasing Activity by Intranasal Administration of a Synthetic Hexapeptide (Hexarelin)

This study was designed to compare the effects of intranasal versus intravenous growth hormone (GH)-releasing peptide (the hexapeptide, His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2, known as hexarelin). Ten children with familial short stature and 2 young adults with GH deficiency were tested. The children with familial short stature had normal GH responses to clonidine and insulin, whereas the GH-deficient subjects failed to show responses to either. The GH-deficient subjects were studied after they had been off human GH for at least several years.

Each subject was given hexarelin twice within 1 week, either intravenously (IV) (1 µg/kg) or intranasally (IN) (20 µg/kg) initially. Blood samples for GH, thyrotropin (TSH), free thyroxine (fT_4), and triiodothyronine (T_3) concentrations were obtained at 0, 15, 30, 60, 90, and 120 minutes.

There were no differences in the mean peak GH response to hexarelin administration depending on its route of administration (79.6 ± 53.1 mU/l IV versus 72.2 ± 35.5 mU/l IN). However, the peak GH concentration occurred approximately 15 to 30 minutes after IV administration, while the peak GH concentration after IN hexarelin occurred 30 to 60 minutes after administration. TSH concentrations fell significantly by 120 minutes, but remained within the normal range. This fall in plasma TSH following hexarelin administration may be the result of partial action on the hypothalamus. There were no significant changes in plasma fT_4 or T_3. The authors conclude that this particular hexapeptide is effective as a provocative test for GH secretion.

Laron Z, et al. *Clin Endocrinol* 1994;41:539-541.

***Editor's comment:** This is a short but important report. GH-releasing peptides (GHRPs) are now being studied for their activity in human subjects. Although the authors of this report suggest that IN hexapeptide would be a good provocative test for GH secretion, the obvious inference is that this compound or a similar synthesized GHRP may someday be useful in treating individuals with defects in GH secretion. Demonstrating that the IN route of administration induces similar GH release as does IV administration strengthens the practicality of these compounds for use in children with GHRP-treatable disorders. The effects of chronic GHRP administration on thyroid function, however, would need to be carefully monitored based on the TSH-lowering effects of hexarelin in the present study.*

William L. Clarke, MD

Estrogen Resistance Caused by a Mutation in the Estrogen-Receptor Gene in a Man

The authors describe a fully virilized, 28-year-old adult male with absence of a functional estrogen receptor. This disorder was characterized by: (*a*) tall stature and continuous linear growth throughout adult life (during childhood height pursued the National Center for Health Statistics 75th percentile while at the age of the report height was 204 cm, or +4.2 standard deviations [SD]); (*b*) unfused epiphyseal growth plates of the long bones and a wrist bone age of 15 years; (*c*) osteopenia; (*d*) increased serum concentrations of luteinizing hormone and follicle-stimulating hormone, and estradiol and estrone with normal levels of testosterone; (*e*) normal sperm number but decreased sperm viability; (*f*) mild glucose intolerance, hyperinsulinism and acanthosis nigricans; and (*g*) lack of effect of high-dose estradiol delivered transcutaneously on sexual characteristics, breast growth, or bone mineral density. The parents of this patient were second cousins. This autosomal recessive trait was associated with substitution of thymine for cytosine at codon 157 in exon 2 of the estrogen receptor gene, resulting in substitution of a stop codon (TGA) for arginine (CGA) at this position and a highly truncated estrogen receptor with no DNA- or hormone-binding domains.

This patient, when compared with his normal siblings and parents, demonstrates that: (1) estrogen activity is not essential for life, fetal development, postnatal growth, or virilization in the male; (2) heterozygous males and females with one defective estrogen receptor allele are normal; (3) estrogen is essential for complete epiphyseal maturation and fusion and for normal skeletal mineralization in the male; (4) estrogen is essential for regulation of gonadotropin secretion in the male; and (5) estrogen may be necessary for normal insulin sensitivity and sperm viability.

Smith EP, et al. *N Engl J Med* 1994;331:1056-1061.

Editor's comment: *This report clarifies earlier reports in which the importance of aromatization of androgen to estrogen in the regulation of gonadotropin secretion in the male had been questioned. Since the level of insulin-like growth factor 1 was normal in this subject, it is possible that the secretion of growth hormone is not dependent on estrogen action. Studies of endogenous and stimulated secretion of somatotropin in this subject would be of interest.*

The phenotype of a homozygous female deficient for the estrogen receptor is unknown, but one might speculate that such an individual may be virilized in utero and during adolescence. Shozu et al[1] and Conte et al[2] report the occurrence of female pseudohermaphroditism and pubertal virilization in females with an abnormality in the gene encoding the P450 enzyme aromatase, leading to decreased estrogen production in utero and unopposed androgen activity. In these respects, females with aromatase deficiency resemble female spotted hyenas who have aromatase deficiency; these females are virilized and quite aggressive.[3]

Allen W. Root, MD

1. Shozu M, et al. *J Clin Endocrinol Metab* 1991;72:560-566.
2. Conte FA, et al. *J Clin Endocrinol Metab* 1994;78:1287-1292.
3. Yalcinkaya TM, et al. *Science* 1993;260:1929-1931.

The Small Nuclear Ribonucleoprotein-Associated Polypeptide N (*SNRPN*) Gene in Prader-Willi and Angelman Syndromes

Imprinting is the process by which differences in the phenotype of a specific disorder are expressed depending on whether the allele was paternally or maternally derived. Imprinting occurs during gametogenesis; it is heritable and reversible.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) map to chromosome 15q11-q13, and they represent 2 of the best examples of imprinting in humans. PWS is characterized by infantile hypotonia, mental retardation, hyperphagia, and small hands and feet. AS is characterized by severe mental retardation, absent speech, seizures, ataxic gait, and bouts of uncontrollable laughter.

In PWS, approximately 70% of patients have a deletion involving the paternally derived chromosome 15; almost all of the rest of PWS patients have maternal uniparental disomy (UPD) of chromosome 15. In contrast to PWS, AS is associated with a similar area of chromosome 15 deletion but on the maternally derived chromosome 15 and with paternal UPD. The study of the molecular similarities and clinical differences between these 2 syndromes has provided valuable information regarding the gene control mechanisms involved in imprinting.

The small nuclear ribonucleoprotein-associated polypeptide N (*SNRPN*) gene has been mapped to the 15q11-q13 region. It is known to display paternal allele-specific expression in mouse and to be expressed exclusively from the father's allele in human fetal brain (Reed et al). Following the localization of the *SNRPN* gene, Sutcliffe et al constructed a complete yeast artificial chromosome (YAC) containing the region commonly deleted in PWS and AS (Lalande) in order to determine the molecular basis for PWS and AS.

Two genes, PAR-1 and PAR-5, were isolated and mapped distal to *SNRPN*. Both PAR-1 and PAR-5 were detected in cultured cells of AS deletion individuals but not in cells of PWS patients, suggesting that these 2 genes are expressed only from the paternal chromosome.

The fact that PAR-1, PAR-5, and the *SNRPN* gene are in close proximity led them to the suggestion that these genes lie in a domain, ie, a group of genes with similar genetic control, of imprinted transcription. The highest levels of expression of *SNRPN* were in brain. PAR-5 expression also was highest in brain, while PAR-1 expression was highest in skeletal muscle. This suggests tissue specificity of gene expression.

Reed ML, et al. *Nat Genet* 1994;6:163-167.
Sutcliffe JS, et al. *Nat Genet* 1994;8:52-58.
Lalande M. *Nat Genet* 1994;8:5-6.

Editor's comment: *Imprinting is increasingly being recognized as a very important molecular mechanism. It appears to be involved in genetic control of growth and behavior, and in early development. Intensive investigation of the 15q12 region is showing important differences in gene expression between the maternally and paternally derived chromosomes. The expression is tissue specific, time-in-development specific, and strain specific.*

Judith G. Hall, MD

Predicting Adult Stature Without Using Skeletal Age: The Khamis-Roche Method

Khamis and Roche developed a modification of the Roche-Wainer-Thissen (RWT) stature prediction model in which the skeletal age was not used to calculate the predicted height. The parameters considered for the calculation of predicted adult height were current height and weight and midparental height, ie, the mean of the parents' heights. They obtained these data from a group of white American children (223 males and 210 females) residing in southwest Ohio; they were participants of the Fels Longitudinal Study and were followed with measurements of height and weight every 6 months from the age of 3 years until 18 years. The stature of each parent also was measured. Linear regressions of adult stature (considered for their purpose as the stature attained at age 18 years) were calculated using the 3 variables. The following equation was used: predicted adult stature = β_0 + β_1 stature + β_2 weight + β_3 midparental stature. The tables for males and females list the intercepts (β_0) and the coefficients of the 3 variables (β_1, β_2, and β_3, respectively) for each chronologic age, expressed in 6- and 12-month intervals. The accuracy of the prediction method was measured using the median absolute deviation (MAD), which is the median of the absolute differences, regardless of the signs, between actual and predicted stature at age 18. The smaller the MAD, the better the accuracy. There was only a slight deterioration of the accuracy with this method as compared with RWT, which uses estimations of skeletal age.

Khamis HJ, Roche AF. *Pediatrics* 1994;94:504-507.

Editor's comment: *The authors present an ingenious method of predicting final adult stature in children without using skeletal age. This method might be a useful adjuvant in the clinic and allows comparisons of predicted adult height by anthropometric determinations. Large discrepancies between the 2 methods may indicate inaccurate measurements and/or inaccurate bone age estimation. Two problems may still preclude its use in a pediatric endocrine setting: first, its accuracy seems to be worse in the peripubertal years, especially in males, where it overestimates predicted heights. Second, the predictability is good only in the absence of pathologic conditions that alter the potential for linear growth.* ***Thus, caution must be exercised if it is used as an adjuvant diagnostic tool in children with short or tall stature.*** *However, it may be of a great value as a descriptive instrument for prediction of adult stature in normal children, and in epidemiologic studies of population when adult height predictors without bone age estimates may be an important index of health status.*

Fima Lifshitz, MD

2nd Editor's comment: *After publication of this article, the authors noted an error in Tables 1 and 2 presenting the weight coefficients necessary for calculating adult stature with the above equation. The decimal point was displaced one space to the left. The weight coefficients may be corrected by shifting the decimal points one space to the right of their present locations. This error is to be corrected in a "Letter to the Editor" of* Pediatrics. *Readers may wish to correct the tables in the original article for their own use or watch for publication of the corrected tables in* Pediatrics.

As the authors point out, the described method for prediction of adult height is based on measurements of healthy white children who are growing normally and, therefore, strictly applicable only to this group. This reviewer seldom predicts stature in normal children, because if the prediction is below that which the parents desire, pressure for intervention—no matter how futile—may be increased.

Allen W. Root, MD

Growth of Short Normal Children in Puberty Treated for 3 Years With Growth Hormone Alone or in Association With Gonadotropin-Releasing Hormone Agonist

Thirty early pubertal short normal subjects received growth hormone (GH) at 0.1 IU/kg/d, 6 d/wk (~0.2 mg/kg/wk) for 3 years. These included 16 males, aged 14.4 ± 0.8 years, and 14 females, aged 12.2 ± 1.2 years. All were at pubertal stage 2 or 3, with slow pubertal growth (4.2 ± 1.2 cm/y) and a mean bone age delay of 2 years. There was no detected GH deficiency or other cause for short stature. Their mean birth length was 48.6 to 49.5 cm at term; the mean of midparental heights was -0.6 to -0.8 standard deviations (SD) below the mean of the general adult population. They were randomized in 2 groups: group A received GH alone; group B received gonadotropin hormone-releasing hormone agonist (GnRHa) plus daily GH injections for 2 years, and for year 3.

The annual growth velocity (GV) increased during the first year in both groups and sexes, the increase being significant (P<0.01) in group A only. The patients of group A maintained an improved GV in the second year, and then returned to pretreatment GV in the third year, while completing their sexual development and bone maturation. Their height, expressed as SD score (SDS) for bone age, improved in the first 2 years but decreased thereafter. Group B patients returned to pretreatment GV in the second year, and demonstrated no significant improvement when treated with GH alone during the third year of the study. They had no significant progress of height for age at any time. Their bone maturation, slow when they were receiving GnRHa, accelerated when sexual development resumed.

At the end of the 3 years, height, expressed as SDS for age, improved in group A from -2.5 ± 0.6 SD to -1.5 ± 0.4 SD in males (P<0.05) and from -2.8 ± 0.5 SD to -2.1 ± 0.9 SD in females (NS). Expressed as SDS for bone age, mean height slightly improved in males (NS) but not in females. In both groups and sexes, the mean predicted height according to Bayley and Pinneau was only slightly increased at the end of 3 years on GH, with a gain of 2 to 5 cm on the average. There was a wide interindividual variability in these results within each group. Pretreatment characteristics of the patients did not account for individual differences. Annual measurement of plasma insulin-like growth factor 1 showed different degrees of increase, not correlated with any parameter of the patients' growth.

The authors drew the following conclusions: (1) Inhibiting sexual development in short early pubertal subjects has no advantage. This was previously demonstrated with GnRHa alone (see *GGH* 1993;9[4]:13), and now is confirmed for GnRHa plus GH. (2) GH alone, at the dose used, can accelerate for

2 years the growth of such slow-growing normal short adolescents, and slightly improve their predicted height in relation to the result of the first year of treatment. However, the expected results should not be overestimated, nor should this be considered as an indication for any routine use of GH in endocrinologically normal and constitutionally short pubertal individuals.

Job JC, et al. *Horm Res* 1994;41:177-184.

Editor's comment: *This report will discourage only the most desperately short children from trying to achieve normal height by using GnRHa plus GH.*

Robert M. Blizzard, MD

Susceptibility Gene Loci for Insulin-Dependent Diabetes Mellitus (IDDM): A Review

Insulin-dependent diabetes mellitus (IDDM) is a polygenic multifactorial disease, ie, it is caused by different susceptibility genes and environmental factors in different people. Other disorders thought to be polygenic multifactorial include ischemic heart disease, asthma, and schizophrenia.

In mice, IDDM has been shown to be a polygenic trait, with the major locus encoded in the major histocompatibility complex (MHC) with at least 10 other loci contributing to the development of the disease. In humans, the MHC HLA region on chromosome 6p21 and the insulin gene region on chromosome 11q23 have been associated with IDDM. However, in families with multiple affected individuals, these 2 loci have been suggested to account for less than 50% of the genetic risk of the disease.

Two recent papers by Hashimoto et al and Davies et al reported genome-wide linkage studies for the localization of IDDM susceptibility loci. Hashimoto et al applied highly informative markers to a panel of 314 white IDDM-affected sibling pairs and found evidence for the localization of a previously undetected susceptibility locus for IDDM in the region of the fibroblast growth factor 3 (*FGF3*) gene on chromosome 11q.

These results were confirmed by Davies et al, who also used the same method of genome-wide searches to study 96 sibpair families and a linkage map of 290. This group also found linkages between IDDM and chromosomes 11q and 6q, and suggested that there may be a fifth susceptibility locus on chromosome 18. Davies et al point out, however, that the genome linkage map had an average spacing of 11 centimorgans (cM) and that gaps still exist in this map. They suggest that in order to detect all the susceptibility loci for IDDM, it may be necessary to test with markers that are only 3 cM apart.

Hashimoto L, et al. *Nature* 1994;371:161-163.
Davies JL, et al. *Nature* 1994;371:130-136.

Editor's comment: *The genome-wide search method has been used for other multifactorial disorders, especially psychiatric disorders (Lander and Botstein.* Genetics *1989;121:185), but has not provided any linkage data so far, much less specific genes. These new methods are very powerful and should hasten the progress of the Human Genome Project effort to identify all 100,000 human genes.*

Judith G. Hall, MD

Prenatal Treatment of Congenital Adrenal Hyperplasia: A Review

Congenital adrenal hyperplasia (CAH), an autosomal recessive disorder, is the most common cause of ambiguous genitalia in females. Ninety percent of CAH cases are caused by 21-hydroxylase deficiency. In order to prevent virilization in utero, maternal glucocorticoid therapy (specifically, dexamethasone) is started immediately after detection and continued throughout pregnancy; this suppresses fetal androgen production.[1-3] Most of the reported cases that were treated early and adequately were born with normal female genitalia.

A recent paper by Wudy et al[4] documents another successful prenatal treatment of CAH. They report a newborn girl born with normal female genitalia after prenatal dexamethasone treatment (the index case in the family was a boy suffering from 21-hydroxylase deficiency). Molecular genetic diagnosis was not available, and prenatal diagnosis relied on amniocentesis with karyotyping and 17 α-hydroxyprogesterone determination. In order to get an accurate amniotic fluid steroid analysis, dexamethasone treatment was suspended for 5 days prior to amniocentesis.

Previous reports have shown that prenatal dexamethasone treatment must begin as early as the 5th to 9th week of gestation, which may be before the diagnosis of a female fetus is made by amniocentesis or chorionic villus sampling (CVS). Both the dosage and temporary suspension of dexamethasone have been controversial issues in prenatal CAH treatment. Some authors suggested that excessive virilization may occur due to a rebound effect.[3] However, Wudy et al's paper has shown that this is not necessarily true. Furthermore, with the advent of molecular diagnosis, interruption of maternal glucocorticoid therapy may not even be necessary. The potential maternal side effects of dexamethasone therapy include development of a cushingoid face, massive weight gain, and marked striae. Hypertension and increased urinary glucose have also been reported.[3] Nevertheless, many families will opt for prenatal therapy for affected female fetuses.

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3. Loeuille GA. *Eur J Pediatr* 1990;149:237-240.
4. Wudy S, et al. *Eur J Pediatr* 1994;153:556-559.

Editor's comment: *Major progress has been made in diagnostic and therapeutic modalities for this common disorder. With the identification of the gene structure and common mutations, more accurate diagnosis is possible both prenatally and at birth. However, therapy must begin before accurate diagnosis of the fetus is possible, since CVS and amniocentesis are contraindicated before the 10th to 11th week of pregnancy.*

Judith G. Hall, MD

GROWTH, Genetics, & Hormones Index for Volume 10, 1994

March 30-April 1, 1995 13th Testis Wkshp, Raleigh, NC. Info: C Desjardins. Tel: 804-982-4310; Fax: 804-924-8785; E-mail: REPROD@VIRGINIA.EDU.

May 12-13, 1995 2nd Intl Wkshp on Thyroid Hormone Resistance, Padua, Italy. Info: Dr P Beck-Peccoz. Tel: 39-2-546-4063; Fax: 39-2-5519-5438.

May 24-28, 1995 Eur Soc for Human Genet Mtg, Berlin, Germany. Info: Amer Soc of Human Genet. Tel: 301-571-1825; Fax: 301-571-1895.

June 14-17, 1995 Endocrine Soc: 77th Ann Mtg, Washington, DC. Info: Endocrine Soc. Tel: 301-941-0200; Fax: 301-941-0259.

June 22-23, 1995 2nd Mtg of Bone Dysplasia Soc, Versailles, France. Info: Organizing Committee, Dr P Maroteaux. Tel: 011-33-1-44-49-44-82; Fax: 011-33-1-45-66-02-86.

June 25-28, 1995 34th Ann Mtg of the Eur Soc for Paediatr Endocrinol, Edinburgh, Scot. Info: ESPE. Tel: 44-41-553-1930; Fax: 44-41-552-0511.

July 17-28, 1995 Mendelian Genet, Bar Harbor, ME. Info: The Jackson Laboratory. Tel: 207-288-3371; Fax: 207-288-5079.

July 29-August 2, 1995 Recent Progress in Hormone Research 51st Conf, Stevenson, WA. Info: Endocrine Soc Mtgs Dept. Tel: 301-941-0200 or 1-800-HORMONE; Fax: 301-941-0259.

July 29-August 3, 1995 The David Smith Morphogenesis Mtg, Big Sky, MT. Info: Dr KL Jones. Tel: 619-294-6217; Fax: 619-291-8938.

August 2-5, 1995 Portland Bone Symp, Portland, OR. Info: OHSU, Continuing Education. Tel: 800-452-1048; Fax: 503-494-3400.

August 19-25, 1995 Intl Human Genet Mtg, Rio de Janéiro, Brazil. Info: Amer Soc of Human Genet. Tel: 301-571-1825; Fax: 301-571-1895.

September 10-15, 1995 11th Intl Thyroid Cong, Toronto, Can. Info: Amer Thyroid Assoc. Fax: 718-882-6085.

September 13-15, 1995 Intl Symp on DHEA Transformation Into Androgens and Estrogens in Target Tissues: Intracrinology, Quebec City, Quebec, Can. Info: Intl Symp on DHEA.

September 17-20, 1995 5th Intl Cong on Hormones and Cancer, Quebec City, Quebec, Can. Info: 5th Intl Cong.

September 27-30, 1995 Molecular and Developmental Biol of Cartilage, Bethesda, MD. Info: Conf Dept. Tel: 800-843-6927 x-324; Fax: 212-838-5640.

October 13-15, 1995 Symp on Advances in Clin Nutrition, Washington, DC. Info: Amer Coll of Nutrition. Tel: 212-777-1037; Fax: 212-777-1103.

October 18-20, 1995 Intl Symp on Growth, Santiago de Compostela, Spain. Info: Profs F Casanueva, C Dieguez, or M Pombo. Fax: 34-81-572121.

October 24-28, 1995 45th Ann Mtg of the Amer Soc of Human Genet, Minneapolis, MN. Info: M Ryan. Tel: 301-571-1825; Fax: 301-530-7079.

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc. The information in this publication reflects the views of the editors and/or contributors and not necessarily those of the grantor or the publisher.

Published by:
Gardiner-Caldwell
SynerMed

405 Trimmer Road
PO Box 458
Califon, NJ 07830

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc.

Robert M. Blizzard, MD
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GROWTH
Genetics & Hormones
Vol. 11 No. 2 June 1995

Magnetic Resonance Imaging in Pituitary Disease

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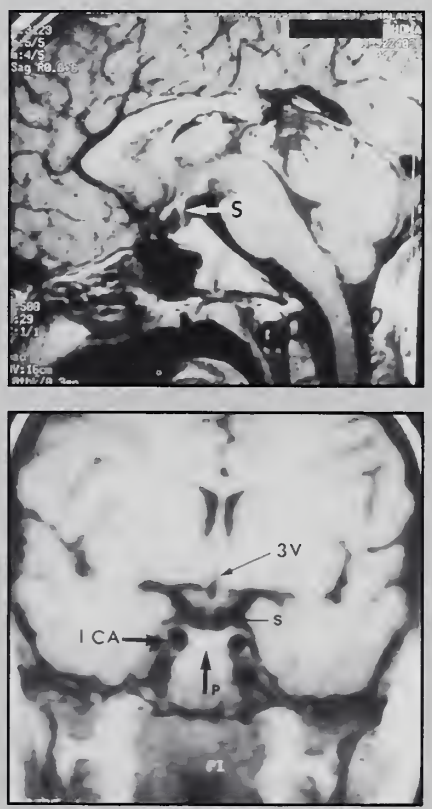
Magnetic resonance imaging (MRI) permits visualization of the anterior and posterior pituitary glands and the pituitary stalk, which connects the pituitary gland to the median eminence. This noninvasive technique has greatly improved our understanding of dysfunction of the anterior pituitary and neurohypophysis in children with growth hormone (GH) deficiency (GHD), idiopathic diabetes insipidus, and central precocious puberty (CPP). This review presents the latest considerations regarding the use of MRI in pituitary disease.[1]

NORMAL MRI OF THE HYPOTHALAMIC-PITUITARY AXIS

The anterior pituitary lobe develops from an upward diverticulum of the primitive buccal cavity. The posterior pituitary lobe, or neurohypophysis, originates as a downward extension from the hypothalamus. The pituitary stalk consists primarily of the neural connection between the median eminence and the posterior pituitary lobe. This neural connection consists of axonal processes down which vasopressin and oxytocin travel to the posterior lobe, from which they are then released by exocytosis.

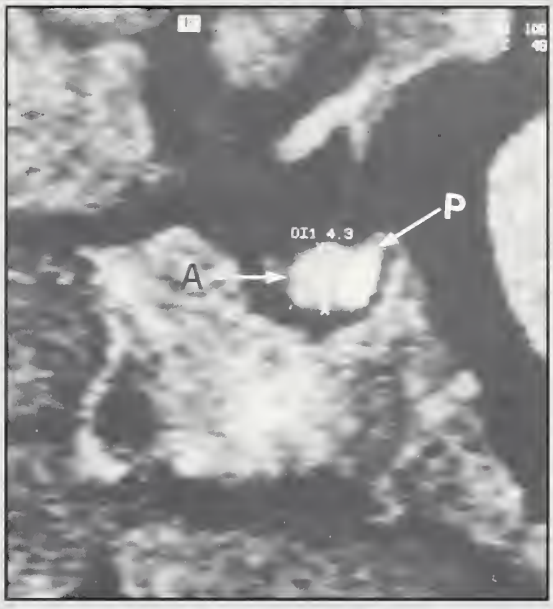
The pituitary stalk consists secondarily of a delicate layer of tissue that is permeated by numerous capillary loops of the hypophyseal-portal blood system. This vascular structure also provides the principal blood supply to the anterior pituitary lobe as there is no direct arterial supply to this organ. In contrast, the posterior pituitary lobe has a direct vascular supply. Therefore, the posterior lobe can be more rapidly visualized in a dynamic mode after administration of gadolinium (gadopentetate dimeglumine) as contrast material during MRI (Figure 1). The use of gadolinium is necessary for optimal study of the pituitary stalk since it contains abundant vascular structures that are not obscured by the blood-brain barrier.

Figure 1
Pituitary Anatomy and Normal MRI Findings



S:pituitary stalk; P:posterior pituitary bright spot; 3V:3rd ventricle; CA:carotid artery

Figure 2
Anterior Pituitary Height Measurement (A) and Posterior Pituitary Bright Spot (P)



Standards for proper imaging have emerged, eg, obtaining sagittal and coronal images that are less than 5 mm in thickness, in order to visualize the pituitary stalk and the posterior pituitary lobe. The maximal height of the anterior pituitary gland is measured in a perpendicular plane to the floor of the sella turcica (Figure 2). Additional axial images using precise midline positioning are necessary to visualize any structural defect of the stalk. The mean anterior pituitary height, when measured using strict midline sagittal T1-weighted sections that are 3 to 5 mm thick, varies according to age. In neonates, the mean height is 4.5 mm, as the pituitary is typically convex.[2,3] After 2 months of age, the superior face becomes flatter and the height decreases to 3 mm. One may consider the lower limit of normal height prepubertally to be 3 mm, as the mean height increases progressively to 5.3 mm. A further increase of pituitary height occurs during puberty.[4] The pituitary stalk also increases in diameter.

The posterior pituitary lobe can be easily distinguished by a round, high-intensity signal in the posterior part of the sella turcica on T1-weighted images (Figure 2). Provided the appropriate MRI sections have been performed, all normal children and adolescents have a bright spot in the posterior pituitary, which serves as a marker of normal neurohypophyseal function. In adults, an age-related decline of 1% per year in detecting this bright spot was reported.[5] Phospholipid components in the neurohypophysis are believed to account for the signal.[6] The location and shape of the posterior pituitary signal may vary slightly by having a ring appearance or an extension along the inferior part of the pituitary stalk. Any process that disturbs the neurosecretory transport or function may lead to an accumulation of high signal intensity material and an obstruction in ectopic positions, such as in the median eminence or along the pituitary stalk cephalad (Figure 3). The ectopia depicted in this dense image, which is associated with pituitary stalk defects, is generally observed in pathologic conditions such as GHD or destruction of the posterior pituitary. However, ectopia has been found on rare occasion in patients with otherwise normal pituitary function and normal imaging findings of the pituitary stalk.[7]

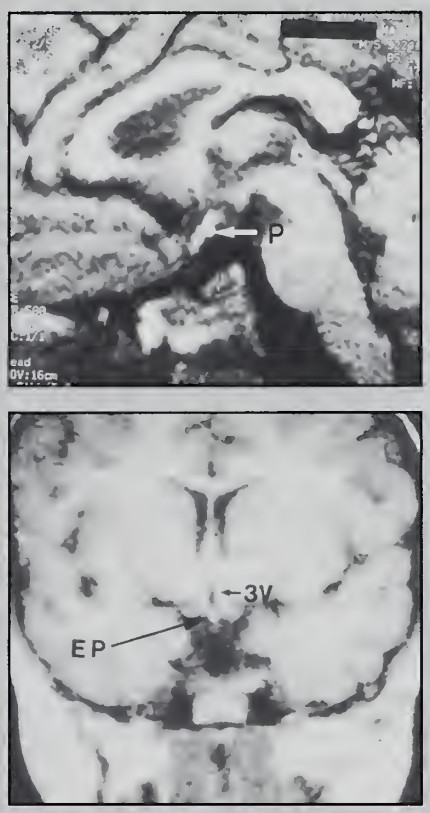
MRI IN ASSOCIATION WITH GHD

Several reports[8-13] have compared MRI and endocrine evaluations of patients with GHD. Pituitary stalk interruption (PSI) with resultant ectopic position of the neurohypophyseal bright spot has been one of the main MRI findings. Hypoplasia of the pituitary gland, with or without ectopic position of the neurohypophyseal bright spot, also occurs. Hypoplasia may occur with a sella turcica of normal or diminished size.

Findings associated with the PSI syndrome are presented in Table 1. Multiple anterior pituitary hormone deficiencies were recently reported in 18 of 29 GHD patients with PSI syndrome, even though diabetes insipidus was absent.[13] Severe hypoplasia of the anterior pituitary with reduced sellar size was present in most. However, a minority of patients with PSI syndrome had apparent isolated GHD. These patients are probably at risk of developing additional pituitary hormone deficiencies in the future, principally at the time of puberty. Additional follow-up data are needed to assess the functional prognostic value of PSI. GHD definitely is more severe in patients with PSI.[14] There is no report of reversal of an abnormal stalk to a normal stalk image.

The pathology of an ectopic neurohypophysis, which is associated with PSI, was first described at autopsy: the tissue of the posterior pituitary lobe was present in a nodule at different levels of the hypothalamicohypophyseal tract. The stalk was reduced to a filament, and the intrasellar pituitary lobe was hypoplastic.[15] This condition is likely caused at times by a pituitary stalk transection, as produced experimentally in animals and observed after pituitary surgery in humans.[16] After pituitary stalk transection, neuroendocrine fibers may regenerate from the hypothalamus. This may explain the fact that diabetes insipidus is usually absent. Pituitary infarction may cause the GHD or multiple pituitary hormone deficiencies. In several studies,

Figure 3
Posterior Pituitary Stalk Interruption



P: ectopic posterior tissue
EP: ectopic posterior pituitary tissue

authors reported the occurrence of significant head trauma in the histories of their patients. This correlated with late onset of growth retardation.[17] Injury also may be of vascular origin because of the structure of the pituitary stalk. Therefore it was appropriate to correlate PSI with the high frequency of perinatal adverse events observed in hypopituitary patients. Inconsistent histories have been obtained and range from significant trauma to perinatal anoxia.[9-13] Events occurring prior to birth also can be considered as a cause of such lesions. For instance, some data suggest that PSI may be associated with midline malformations, a developmental defect occurring before the pituitary is fully formed in patients, such as those with septo-optic dysplasia or a single incisor (Table 1).

Anterior pituitary hypoplasia in the absence of other MRI abnormalities has been reported with GHD, including isolated GHD. Therefore, measurement of pituitary heights is an important method of evaluating pituitary function. The age-related pediatric reference data cited above[3] provides important normal data for pituitary height and, hence, volume.

Patients with only hypoplasia of the pituitary, demonstrated by MRI, tend to have less severe GHD than those with PSI. In a study of 21 children with isolated GHD, the pituitary stalk was intact in all.[18] The anterior pituitary lobe was hypoplastic in 17, and the sella was partially empty in 13. None of these children had a history of perinatal asphyxia. The authors postulated that an embryonic defect was the most likely cause.

Because there are multiple different appearances of the hypothalamic-pituitary axis in association with GHD, the results of MRI may assist in classifying the type of GHD. A prospective comparison of functional and anatomic data in GHD patients remains to be undertaken. In Table 2 (page 4) the etiologies of idiopathic GHD are listed. MRI can assist in differentiating the 4 major groups of GHD considered in the table. In addition, imaging of the pituitary and pituitary stalk can be a valid diagnostic tool when infantile GHD is suspected but not proven by the usual testing techniques.

MRI OF PATIENTS WITH CENTRAL DIABETES INSIPIDUS

The round, high-intensity signal seen in the normal posterior pituitary lobe is usually absent in patients with central diabetes insipidus. However, this signal is present in children with dipsogenic polyuria or nephrogenic diabetes insipidus, or in those with autosomal dominant familial cases of central diabetes insipidus.[19] MRI remains the best technique with

Table 1
Pituitary Stalk Interruption Syndrome (PSIS)

- PSIS is strongly associated with multiple pituitary hormone deficiency, which is associated with organic hypopituitarism. When associated with isolated GHD, it is a valuable diagnostic sign of a permanent defect and may be predictive of later occurring anterior pituitary hormone deficiencies
- PSIS is frequently associated with injury at birth
- Several likely causes of PSIS are:
  - Prenatal developmental defect
  - Perinatal insult
  - Postnatal trauma
- The variable clinical expression of PSIS may reflect a progressively diminishing hypothalamic trophic control with a predominant effect on GH secretion

Table 2
Etiologies of Idiopathic Growth Hormone Deficiency

- Molecular defects of growth hormone or growth receptor genes
- Developmental defects
  - Somatotropic cell differentiation (pituitary factors)
  - Anterior pituitary development (midline defects)
- Pituitary stalk interruption
  - Prenatal vascular compromise or trauma
  - Perinatal asphyxia or trauma
  - Postnatal trauma or infection
- Defective hypothalamic control with isolated anterior pituitary hypoplasia

which to evaluate the pituitary stalk and infundibulum in patients with idiopathic polyuria. A finding such as thickening of the pituitary stalk may be helpful in determining the etiology of apparent diabetes insipidus.[19] Such thickening may be isolated. The degree of thickening is best evaluated after administration of gadolinium. When thickening and/or enlargement is marked, the question of an infiltrative process destroying the neurohypophyseal tract must be considered. Malignant tumors such as germinomas of the hypothalamus must be considered. Our experience is that if such a germinoma is not detected at the time of presentation, it may be recognized within a few months or years by repeat MRI. Measurement of circulating tumor markers such as the beta unit of human chorionic gonadotropin (hCG-β) and α_1-fetoprotein also is indicated.[20] Surprisingly, some patients present with an isolated thickening of the pituitary stalk that may remain stable over years and eventually regress without either any change in MRI findings or the appearance of a tumor. In most, diabetes insipidus remains isolated and normal anterior pituitary function persists. The absence of associated systemic abnormalities (cutaneous, skeletal, pulmonary) and/or hypercalcemia rules out organic infiltration due to histiocytosis, sarcoidosis, or tuberculosis.

Diabetes insipidus may be isolated or associated with GHD (Figure 4). The association of diabetes insipidus with GHD is usually considered as a result of invasion by a craniopharyngioma or by histiocytosis X.[21] However, diabetes insipidus and GHD may be present without evidence of infiltration of any type. In such cases GHD may be variable and, if prolonged, produce growth retardation.[22] Indirect evidence for autoimmune,neurohypophysitis, based on the presence of autoantibodies to vasopressin cells, was suggested,[23] but no follow-up of these patients has been reported. More recently, lymphocytic infundibuloneurohypophysitis was described as a cause of central diabetes insipidus. Histologic evidence was obtained in biopsy specimens from adults in this series. Impairment of GH secretion was often associated. The natural course of this condition was unique with possible regression of the stalk width. There was a preponderance of females, and the authors considered this compatible with the autoimmune hypothesis of idiopathic diabetes insipidus with the presence of T-cell infiltration.[24] Whether these findings apply to diabetes insipidus in the pediatric population remains to be demonstrated. For practical purposes, it may be recommended to rigorously follow up patients with combined diabetes insipidus and GHD with repeat MRI every 6 to 12 months for a period of 2 to 4 years, as the appearance of a dysgerminoma should not be missed. After 2 to 4 years, a repeat MRI every 2 to 3 years is adequate.

MRI OF PATIENTS WITH CPP

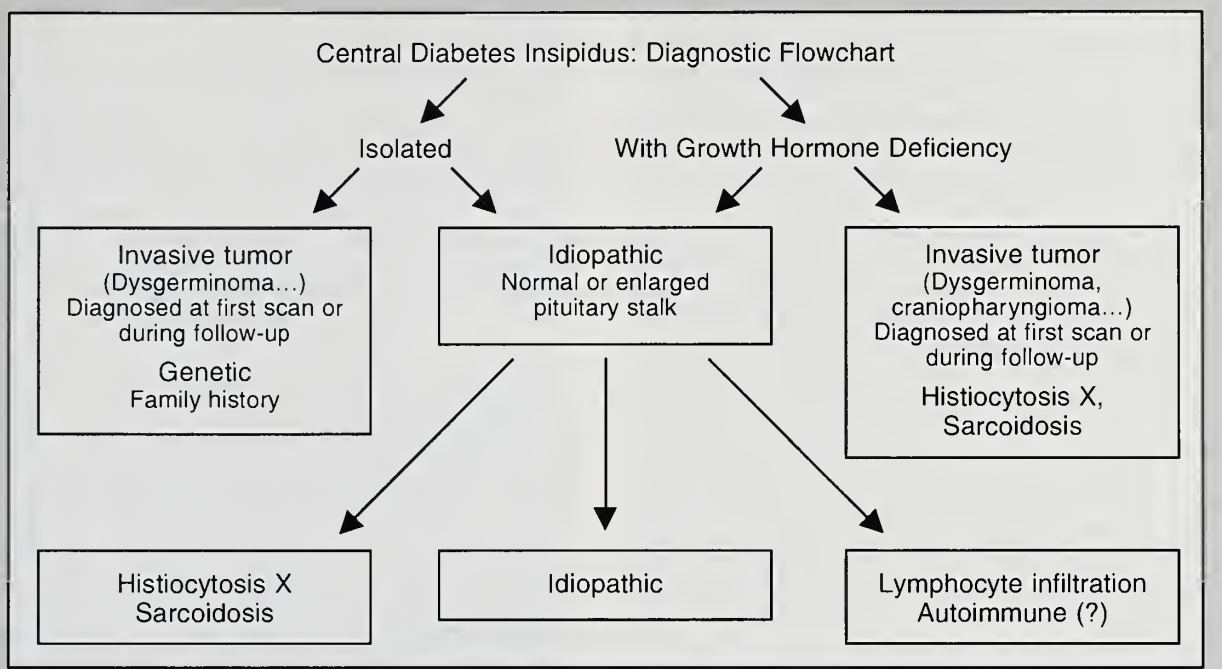
MRI should be pursued in patients with CPP. Invasive tumors like optic and hypothalamic gliomas and congenital malformations such as hydrocephalus and hamartomas may be easily identified. The latter often produce luteinizing hormone-releasing hormone (LHRH), which accounts for the sexual precocity. Except for extensive hCG-β–secreting dysgerminomas in boys extending into the pituitary sella, which are invariably associated with diabetes insipidus, there are no invasive intrasellar lesions causing CPP. However, the pituitary gland undergoes transient hypertrophy during normal puberty. The height of the anterior pituitary lobe increases with convexity of its upper surface; this should not be mistaken for a tumor. Similar changes occur in patients with CPP.[4] However, small pituitary glands with markedly concave upper borders were observed in patients presenting with associated GHD and sexual precocity.[25]

SUMMARY

MRI is essential in the evaluation of any patient with suspected pathology of the hypothalamic-pituitary axis. Physicians involved in the care of such children should not hesitate to refer such patients to the appropriate specialists. The morbidity and mortality statistics will be improved significantly.

ACKNOWLEDGMENTS
The author is grateful to Drs. R. Brauner, F. Brunelle, and M. Argyropoulou for their critical contribution and Mrs. C. Castanera for her skillful assistance.

Figure 4



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Abstracts From the Literature

Is the Etiology of Insulin-Dependent Diabetes Mellitus Related to Superantigen Involvement?

The etiology of insulin-dependent diabetes mellitus (IDDM) remains obscure, although most accept that this is usually a T cell–mediated autoimmune disease, the onset and/or progression of which is very possibly triggered by unknown environmental factors (possibly viruses) acting on a predisposing genetic background. Conrad et al studied the islet-infiltrating T (IIT) cells from 2 IDDM patients who were dying at the onset of disease. Their results can be interpreted as providing evidence for the involvement of a pancreatic islet cell membrane-bound superantigen (SAg) as an etiologic factor.

Conrad et al report a correlation between the presence of insulitis and the presence of an abundance of $CD4^+$ (helper cells). $CD8^+$ (killer cells) were also present in the areas of insulitis. In another article in the same issue of *Nature*, MacDonald and Acha-Orbea comment upon the definition and function of SAgs. The foundation for their speculation is based primarily upon a unique property of SAgs—ie, their ability to activate a large population of T cells in a given person by interacting specifically with amino acid sequences on the "variable" domain (V) of the β chain (β) of the T-cell receptors (TCRs), and, therefore, the TCR Vβ and, specifically in the instance of insulin producing cells, the TCR Vβ7 receptor. Conrad et al demonstrated a strong overexpression of the Vβ7 family of cells in the IIT cells from the 2 patients studied, suggesting the presence of a SAg that triggers preferentially Vβ7 and T cells, rather than conventional Ags, in the etiology of IDDM.

SAgs are unique products of ubiquitous bacteria and viruses. They postulated that if the first exposure to the SAg is at a very

early age, potentially autoreactive T-cell clones are inactivated. The silencing of such "dangerous" T-cell clones may then protect against the development of IDDM. If instead the first exposure is several years after birth, many different T-cell clones expressing the same TCR Vβ will become simultaneously activated; and in a genetically predisposed individual, some of these T cells are able to initiate the process that eventually results in the destruction of the β cells of the pancreas.

MacDonald and Acha-Orbea discuss 2 possible theses for how the polyclonal T cell–activating property of SAg could be responsible for the onset of a specific autoimmune disease such as IDDM. Readers who are interested in these alternatives are referred to the excellent presentation by MacDonald and Acha-Orbea, who point out that all SAgs identified to date are the products of either bacteria or viruses. This raises the obvious possibility that the IDDM-associated SAg, if it exists, is of infectious origin. These commentators further state that although the arguments advanced by Conrad et al in favor of SAg involvement in IDDM are thought provoking, they should be hedged with caveats. First, the authors have data on only 2 rather unusual IDDM patients who died rapidly after the onset of disease; and second, neither the SAg nor its putative causative agent has been identified.

Conrad B, et al. Evidence for superantigen involvement in insulin-dependent diabetes mellitus aetiology. *Nature* 1994; 371:351-355.

MacDonald HR, Acha-Orbea H. Superantigen as suspect. *Nature* 1994;371:283-284.

Editor's comment: *The concepts presented above are exciting to consider. Thirty-four years ago in 1961, my collaborators and I postulated that IDDM was an autoimmune disease in many instances. This theory, although slow in being accepted, has now been accepted for approximately 20 years. However, the causative factors in the autoimmune process remain unclear. Pursuit of the SAg hypothesis is essential. Recently, some of us received a letter from Dr. Dorothy Becker, in which she requested your potential collaboration in helping elucidate further the possible role of SAgs in the etiology of IDDM. Because your assistance in this elucidation is important, I have invited Dr. Becker to add her own comment below.*

Robert M. Blizzard, MD

2nd Editor's comment: *It is now clear that the development of IDDM in animal models and the majority of humans with the disorder is an autoimmune process that develops in genetically susceptible individuals. Our group has sought an environmental trigger for the induction of this process for the past 15 years. Work from Pittsburgh, as well as that from many groups around the world, has shown epidemiologic associations with a variety of viruses and food components. However, rigorous examination has continually failed to elicit a clear association of IDDM with any one environmental agent. The relatively recent explosion in the application of immunologic techniques to IDDM research and the availability of pancreatic tissue from new-onset IDDM children have allowed the proposal of the SAg theory in the etiology of IDDM described above. As SAgs have been invoked as causative agents in other autoimmune diseases, this theory has some precedent. If a SAg could be proved to be an initial trigger or a subsequent "hit" that either induces or allows the continued progression of the autoimmune process, IDDM theoretically could be prevented by antibiotic treatment of the agent (such as streptococcal disease) or vaccination against the agent. We therefore feel that this avenue of research has to be pursued as we continue our efforts to ultimately prevent the onset of IDDM in children. Fortunately, any given center in the United States does not experience frequent mortality in children with IDDM, which, unfortunately, leads to a major lack of availability of material with which to work. Therefore, Dr. Massimo Trucco and I have requested the assistance of pediatric endocrinologists and pediatricians around the country in obtaining fresh pancreatic material from any individual who might die at the onset of IDDM. In addition, it is important to investigate individuals from different regions of the country to ensure that Dr. Trucco's findings in 2 children who came from the same area are applicable over a wider geographic region. We feel that Dr. Trucco's work in the immunogenetics division of the Children's Hospital and University of Pittsburgh is extremely exciting, and we hope we can get the assistance and support of pediatricians around the country, which would allow its rapid continuation and progress. Dr. Trucco can be reached at (412)692-6570, or one of our colleagues can be reached at any time through the operator at Children's Hospital of Pittsburgh, (412)692-5325.*

Dorothy Becker, MD

Cognitive Abilities Associated With the Silver-Russell Syndrome

The developmental status of 25 children between 6.0 and 11.8 years of age (20 males, 5 females) with the Silver-Russell syndrome was evaluated. Based on assessment of the father's occupation, more than half of the children were from middle class and upper socioeconomic groups and the ascertainment bias of sample would, if anything, be expected to have identified children with above average abilities. Of the 25 children, only 3 (12%) had full-scale IQ scores above average (IQ >116 to 130); 9 (36%) scored within average range of abilities (IQ 85 to 115); 5 (20%) had scores in the range of borderline mental retardation (IQ 70 to 84); and 8 (32%) had scores in the range associated with mild to moderate learning disability (IQ <70). There was little variation between the mean full-scale IQ of 85.9 ± 23.7 (using the Wechsler Intelligence Scale for Children [WISC]), the mean verbal IQ of 89.3 ± 22.6, and the mean performance IQ of 84.3 ± 23.5. The authors reported that mean performance IQ scores were lower in girls than in boys; however, the number of females (n=5) studied was small.

The IQ scores correlated best with head circumference measured at the time of the test. The 3 children with superior IQ scores all had normal head circumferences for chronologic age. Utilizing the Neale analysis of reading ability, the mean reading comprehension was delayed relative to chronologic age by 15.4 months, accuracy by 14.4 months, and rate of reading by 13.8 months. Twelve of the 25 children required special education or remedial assistance.

The study indicates that as a group, children with the Silver-Russell syndrome have an average IQ that is 1 standard deviation (SD) below that of the general population; one third of these have a developmental ability classified within the learning disability range.

Fifteen of the children were treated with growth hormone (GH). The mean change in height SD score between diagnosis and testing was +1.97 in GH-treated children and 0.32 in untreated children, suggesting that GH increased the growth of the treated children. Data concerning bone age advancement and similar parameters were not reported as part of the study.

Lai KYC, et al. *Arch Dis Child* 1994;71:490-496.

Editors' comment: *These data will be useful in counseling the parents of children with this syndrome and in planning early educational intervention for those children who require remediation. Surprising is the fact that there is as much mental retardation and learning disability as is reported here. Other investigators have alluded that mental retardation might be part of the syndrome upon occasion, but the data have been exceedingly limited. This, of course, was why the study was undertaken by the authors and why it is of significant value. Differences found between the males and females, however, must be interpreted cautiously because of the very small number (5) of females in the study.*

Surprisingly, 4 of 18 of the children were found to have concomitant GH deficiency (GHD). GHD has been recognized in the past as occurring very occasionally in the Silver-Russell syndrome, but there are probably not more than 6 to 10 cases in the world's literature of patients with Silver-Russell syndrome having GHD. At least one of those had an associated craniopharyngioma. The authors' reports concerning the results of GH therapy are very limited in this article. The patients reportedly did have an increase in the mean change in height SD score of +1.97. No reference is made as to whether the patients treated included any or all 4 of the patients with GHD. There is no discussion of the therapeutic regimen, nor of the advancement or delay in bone age, or, therefore, any potential change in predicted height. In our opinion, this portion of the presentation should have been omitted because it suggests that GH treatment may be effective in patients with Silver-Russell syndrome when no data are given to permit evaluation of that possibility. We have repeatedly observed that patients with Silver-Russell syndrome treated with GH grow at an increased rate for the first 1, 2, or 3 years; frequently, however, the patients have marked slowing of growth while on GH and proceed to grow at a rate less than the pretreatment rate—even though GH is given at progressively increased doses. We rush ahead to say that our impressions are exactly that, impressions, and an inadequate number of patients have been given treatment over a period of 7 to 10 years to permit evaluation of the true response to GH treatment. Such studies do need to be done.

Allen W. Root, MD, and Robert M. Blizzard, MD

Molecular Basis of Mammalian Sexual Determination: Activation of Müllerian Inhibiting Substance Gene Expression by SRY

The pathway of male sexual development in mammals is initiated by *SRY*, a gene on the short arm of the Y chromosome. Its expression early in the differentiating gonadal ridge directs testicular morphogenesis from the bipotential gonadal anlagen. The testis then produces testosterone from the Leydig cells and müllerian inhibiting substance (MIS) from the Sertoli cells. The latter prevents the müllerian system from developing into a uterus and fallopian tubes. There is a gene for SRY and a gene for MIS. Functional studies of SRY in a cell line taken from the embryonic gonadal ridge revealed that its activation leads to expression of MIS. SRY molecules containing mutations producing human sex reversal have altered structural interaction with DNA and fail to induce transcription of MIS.

The molecular mechanisms of interaction between SRY protein, DNA, and the target gene or genes regulated by SRY have not been identified. The investigators did demonstrate that there is a connection between SRY and MIS, and they provide evidence for an intervening factor or factors, which are designated SRYIFs and which are interposed in the action between SRY and the *MIS* promoter. Their studies permitted them to conclude that SRY induces expression of the human *MIS* promoter in vitro. Mutation at SRY-I68 abolishes the transcriptional response of the *MIS* promoter to *SRY.* Although mutation of the *MIS* promoter region itself diminished binding of *SRY* to the promoter, there was no diminished transcriptional response of the *MIS* promoter to SRY in the cell line used in vitro. This suggests that SRY-dependent transcriptional activation of the *MIS* promoter is indirect, and perhaps occurs through postulated SRYIFs, which may be the primary target genes of SRY.

Haqq CM, et al. *Science* 1994;266:1494-1500.

Editor's comment: *Readers who are intrigued with sexual differentiation will find this article most elucidating. The data presented are important for their concepts. The reviewer suggests that this article be read in conjunction with a review by Bogan and Page, entitled "Ovary? Testis? — A Mammalian Dilemma" (*Cell *1994;76:603-607).*

Dr. Alfred Jost started the story rolling in 1953 when he discovered that the presence of testes during mammalian embryogenesis results in male differentiation of both the internal sex organs and external genitalia. The story is obviously still rolling. Today's findings are as exciting in explaining the mechanisms of sexual differentiation as were Dr. Jost's.

Allen W. Root, MD

In a Future Issue

The Neuroendocrine Landmarks of Puberty
by Jean-Pierre Bourguignon, MD, PhD

Family History of Alzheimer's Disease May Increase Risk of Birth of Children With Down Syndrome

A progressive neuropathy is seen in adult individuals over the age of 40 with Down syndrome (DS) that is similar to that seen in Alzheimer's disease (AD).[1] The brain pathology seen in both disorders has led to the suggestion that DS and AD may be genetically related. Heston et al[2] discussed an association between the incidence of DS and AD in some families. Van Duijn et al[3] reported an increased frequency of DS births in the families of individuals with AD, as well as an increased incidence of AD in relatives of individuals with DS.

In a recent report by Schupf et al,[4] the parents' history of dementia was examined in families of 96 adults with DS and 80 adults with other forms of mental retardation. Schupf et al postulated that since most of the nondisjunction events leading to trisomy 21 in DS are maternal, there would be an associated increased frequency of AD among mothers but not fathers of individuals with DS.

They studied the families of 96 adults with DS and separated the groups of mothers into those who gave birth at over the age of 35 and those under the age of 35. An increase in dementia was found among the mothers of DS probands in both groups when compared with the mothers of individuals with other types of mental retardation. This increase was more significant in the younger group (<35 years) than in the older group of mothers. Schupf and colleagues suggest that familial aggregation of dementia among mothers of adults with DS supports the hypothesis of genetic susceptibility to both disorders.

After the age of 35, the risk for having a child with DS increases. In mothers under the age of 35, the incidence of DS births is much lower. Schupf et al suggest that a susceptibility factor probably related to accelerated aging may be playing a role in the birth of individuals with DS in the study cohort of mothers under the age of 35. They also suggest that a family history of AD should be considered as a risk factor for DS birth, especially in women under the age of 35.

1. Wisniewski KE, et al. Alzheimer's disease in Down syndrome: clinicopathological studies. *Neurology* 1985;35: 957-961.
2. Heston LL, et al. Dementia of the Alzheimer type: clinical genetics, natural history and associated conditions. *Arch Gen Psychiatr* 1981;38:1085-1091.
3. Van Duijn CM, et al. Familial aggregation of Alzheimer's disease and related disorders: a collaborative reanalysis of case-control studies. *Int J Epidemiol* 1991;20(suppl 2): S13-20.
4. Schupf N, et al. Increased risk of Alzheimer's disease in mothers of adults with Down's syndrome. *Lancet* 1994;344: 353-356.

Editor's comment: *Over the last few years, research has led to the recognition of different susceptibility factors for a number of genetic disorders. The report by Schupf et al is interesting in that an association between a chromosomal disorder and a neurologic disorder has been made. These 2 conditions are related by the histopathologic findings of the brain. However, with an increased risk for DS in the AD families, other causative factors may be found. This may be just a scratch on the surface of other possible associations. The next question to explore is what causes the susceptibility and whether it can be modified.*

Judith G. Hall, MD

Neurofibromatosis Type 1 Due to Germ-Line Mosaicism in a Clinically Normal Father

Two siblings with neurofibromatosis type 1 (NF1) were born to normal parents. There was no family history of this disease. Both had a 12-kb deletion of the *NF1* gene, which is located on chromosome 17; *NF1* genes in the lymphocytes of both parents were normal. Nevertheless, the intragenic deletion of *NF1* in the patients was of parental origin. Analysis of DNA from the father's spermatozoa revealed that 10% of the sperm had the typical *NF1* deletion that was present in his affected offspring. This father had an isolated mutation in *NF1* confined to a small number of germ cells.

Lázaro C et al. *N Engl J Med* 1994;331:1403-1407.

Editor's comment: *The* NF1 *gene encodes a peptide known as neurofibromin that is homologous to* ras *guanosine triphosphatase. Approximately 50% of patients with NF1 arise from fresh mutations; in 90% of the patients with a sporadic mutation in* NF1 *the mutation has occurred in the paternally derived* NF1 *allele. The data presented in this abstract illustrate the role of postzygotic mutation, which may lead to somatic and/or germ-line mosaicism in the cells programed for gamete formation (Bernards A, Gusella JF.* N Engl J Med *1994;331:1447-1449). Since spermatogenesis is an ongoing and active process, errors might be expected to occur more commonly in genes of paternal than of maternal origin. One wonders if the incidence of male germ-line mosaicism may be greater than suspected.*

Allen W. Root, MD

The Mouse Obese Gene and Its Human Homologue

The structure of the *ob* gene in mice, which is associated with obesity and type II diabetes mellitus, was determined. The mouse *ob* gene codes for a 167 amino acid peptide. Amino acids 1 to 21 are likely to be a signal sequence, suggesting that the mature protein has 146 amino acids. In accord with the thesis that adipose tissue secretes a substance acting upon the hypothalamus to suppress appetite, messenger RNA of the *ob* gene is expressed only in white adipose tissue in the mouse. In the homozygous *ob/ob* mouse, a mutation in codon 143 prevents translation of this gene product. The *ob* gene homologue is found in the rat, rabbit, vole, eel, sheep, pig, and cow and in humans. The human homologue of *ob* gene product also has 167 amino acids and is 84% homologous with the mouse gene product. The hypothesis examined by Zhang et al[1] is that this peptide is a product of the fat cell and that it is secreted and serves as a negative feedback signal to the ventromedial nucleus of the hypothalamus, thereby establishing a homeostatic mechanism for caloric intake and energy utilization.

Rink,[2] in an editorial appearing in the same issue of *Nature*, provides an excellent review of the current concepts pertaining to the possibility that such a protein exists. He states that localized damage to the hypothalamus, which is the main control center for satiety and energy expenditure, produces obesity similar to that observed in the genetic *ob/ob* mouse. He also recounts that rats forced to overeat lay down excessive fat, but when offered a normal diet, they eat less until their normal body weight is restored; and removal of a substantial mass of fat is followed by extra eating, which increases the remaining fat stores. Rink also states that the data of Zhang et al support the concept of a fat-derived satiety factor, which is the most promising hypothesis of several that have been proposed. The postulated *ob* protein is likely to be a fat-derived molecule with a long half-life that acts on the hypothalamus to exert long-term overriding control of appetite and, most likely, fuel storage and energy expenditure.

Some cases of morbid obesity in humans may reflect a homozygous condition analogous to that in the *ob/ob* mouse where the protein is not produced. The more common forms of obesity might reflect subnormal production of the protein.

1. Zhang Y, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425-432.
2. Rink TJ. In search of a satiety factor. *Nature* 1994;372:406-407.

Editor's comment: *The theories concerning the regulation of obesity include: (1) lipostasis, which is the synthesis and secretion by fat of an agent that inhibits appetite at the level of the hypothalamus; (2) glucostasis, which is a theory that blood glucose values regulate the body energy stores by acting on the hypothalamus; (3) body temperature control of energy utilization and fat storage; and (4) dilution of a hypothetical fat-soluble factor that inhibits feeding. Under this thesis, the greater the body fat mass, the greater the storage of this lipophilic agent, which theoretically lowers its circulating levels and lessens its inhibitory influence on feeding. The reported data do lend support to the lipostasis theory, although the secretion, biologic activity, and mechanism of action of this agent have not been determined.*

There are 6 genes that have been associated with obesity in the mouse, which means that much more work is necessary to determine the role of all of these, and possibly other, genes. The biologic activity of the ob/ob *protein and its pattern of regulation of secretion also need to be determined before therapeutic approaches become evident in the clinic. If this gene product proves to be a satiety factor, an exciting period of experimental observations and therapeutic effort is beginning.*

There is much need in this area. Zhang et al have opened the door for this research.

Allen W. Root, MD

Estrogen Levels in Childhood Determined by an Ultrasensitive Recombinant Cell Bioassay

An extraordinarily sensitive (0.02 pg/mL estradiol equivalence) bioassay for the measurement of serum/plasma concentrations of estrogen was developed by the investigators. The unknown plasma and standard samples of estradiol are incubated with transformed yeast. An increase in activity of β-galactosidase is determined to measure the response of estrogen in a sample. Overexpression of the estrogen receptor accounts for the extreme sensitivity. Other factors contribute. Specificity for estradiol is surprisingly great, and variants such as ethinyl estradiol, estradiol sulfate, estradiol glucuronide, estrone, estriol, and diethylstilbestrol are recognized only to a slight extent (<3%). At an estradiol concentration of 2 pg/mL, the intra-assay and interassay coefficients of variation were 15% and 13%, respectively.

In 21 prepubertal girls, aged 5.5 to 10.5 years, serum estradiol concentrations measured by the assay were 0.6 ± 0.6 pg/mL estradiol equivalents, with a range of <0.02 to 2.2 pg/mL. In 23 prepubertal boys, aged 4.5 to 13.0 years, the concentrations were measured at 0.08 ± 0.2 pg/mL, with a range of <0.02 to 0.7 pg/mL. Thus, bioactive estradiol levels were substantially greater in prepubertal females than males ($P<0.05$).

Klein KO, et al. *J Clin Invest* 1994;94:2475-2480.

Editor's comment: *This bioassay is 100-fold more sensitive than the most sensitive of established radioimmunoassays for estradiol. Its specificity for estradiol was unexpected but is exceedingly useful since estradiol is the principal endogenous estrogen in children and adolescents. The higher levels of estrogen in prepubertal girls than in boys, as determined by this assay, may explain some of the variations in the growth patterns of the 2 sexes, such as earlier onset of the growth spurt in girls, earlier pubertal maturation of the hypothalamic-pituitary axis, and more rapid advancement of skeletal maturation.*

The assay is technically demanding and tedious, but offers promise for the evaluation of the dynamics and regulatory controls of estrogen secretion in infancy, childhood, and early adolescence. An assay with this sensitivity has long been needed.

Intriguingly, previously unanswered questions in all probability will now be answered. Application of this methodology to measurement of other substances present in small amounts in other body fluids is anticipated. The importance of this article is 2-fold: (1) it sets the precedent for a new type of assay for measuring exceedingly small quantities of substances in fluids; and (2) estrogen is measured in serum at levels never before attainable. Readers may wish to refer to the original article for details concerning the methodology. Congratulations Dr. Klein and collaborators.

Allen W. Root, MD

Identical Mutations in the *FGFR2* Gene Cause Both Pfeiffer and Crouzon Syndrome Phenotypes

Pfeiffer and Crouzon syndromes are 2 well-characterized autosomal dominant malformation syndromes. Both exhibit craniosynostosis, or premature fusion of the skull bones. Patients with Pfeiffer syndrome also manifest digital abnormalities, including broad, medially deviated great toes and thumbs and variable degrees of syndactyly or brachydactyly of other digits. Although subtle differences exist in the craniofacial features, it is the presence or absence of digital abnormalities, which typically breeds true in families, that distinguishes the 2 syndromes.

Very recently, mutations in the fibroblast growth factor receptor 1 *(FGFR1)* gene have been found in Pfeiffer syndrome and mutations in the *FGFR2* gene have been identified in Crouzon syndrome. The mutations map to similar locations in the respective genes. Surprisingly, Rutland et al now have demonstrated that both Pfeiffer and Crouzon syndromes can result from *FGFR2* mutations. Five patients with Pfeiffer syndrome had a mutation identical to one found previously in a patient with Crouzon syndrome, and 1 patient with Pfeiffer syndrome had the same mutation detected in 3 cases of Crouzon syndrome. Furthermore, the amino acid substitutions that resulted involved residues immediately adjacent to one another: Cys 342 and Pro 341.

These observations raise interesting questions about how the same mutations can give rise to 2 syndromes that have been considered distinct. This article and an accompanying editorial discuss several possibilities. One is genetic variation in other loci whose gene products interact functionally with the mutant *FGFR2* gene product. The interaction between fibroblast growth factors and FGFRs is known to be very complex. Another possibility is a genetic variation in the second "normal" *FGFR2* allele or a variation occuring at another location in the same "mutant" *FGFR2* allele.

Rutland P, et al. *Nat Genet* 1995;9:173-176.

Editor's comment: *Ignorance is a wonderful thing. In the absence of facts it is easy, even fun, to speculate about how diseases come about. Indeed, as pointed out in Mulvihill's editorial, the classic genetic concepts of variable expressivity and pleiotropy have served us well to explain phenomena such as reported here. Differences in genetic background also were commonly invoked to explain such observations. However, as knowledge chips away at ignorance, our explanations must be revised accordingly. The findings reported here provide an excellent opportunity to explore specific mechanisms by which identical mutations can produce seemingly different clinical syndromes. In any event, it is now clear that signaling through the* FGFR2 *receptor is very important to both craniofacial and limb development and that alterations in the extracellular domains of this receptor protein can lead to well-defined malformation syndromes.*

William A. Horton, MD

Mulvihill JJ. Craniofacial syndromes: no such thing as a single gene disease. *Nat Genet* 1995;9:101-102. Editorial.

Deconvolution Analysis of Spontaneous Nocturnal Growth Hormone Secretion in Prepubertal Children With Preterminal Chronic Renal Failure and With End-Stage Renal Disease

Tönshoff et al studied spontaneous nocturnal growth hormone (GH) secretion in 12 children with end-stage renal disease (ESRD) and in 11 children with preterminal chronic renal failure (CRF), and in a control group of 12 matched children with idiopathic short stature (ISS). All subjects were prepubertal. The children with ISS were defined by normal plasma GH responses to pharmacologic stimulation and height ≤2 standard deviations (SD) below age- and sex-matched normative values and the exclusion of any endocrine or other metabolic disorders. The children with preterminal CRF were defined as those with a glomerular filtration rate (GFR) of <70 mL/min/1.73m^2. Children with ESRD were on regular continuous ambulatory peritoneal dialysis. All had growth retardation defined as a height SD score for chronologic age ≤-2 and a height velocity SD score for chronologic age ≤0. The primary renal disease in these children was renal dysplasia/hypoplasia (n=8) and chronic glomerulopathy (n=6). Patients with renal disease were receiving vitamin D, water-soluble vitamins, oral phosphate binders, and oral sodium bicarbonate; they did not receive glucocorticoids, immunosuppressants, or clonidine.

All subjects underwent blood sampling (0.5 mL) every 20 minutes for 10 hours, beginning at 2000. Multiparameter deconvolution analysis was used to determine the number, duration, amplitude, and mass of GH secretory bursts, and to estimate the subject-specific GH half-life in children with preterminal CRF and ESRD. Data were reported as means

± standard error of the mean (SEM) and nonparametric tests were used to determine statistical significance.

Age, weight, height, height velocity, and body mass index did not differ between ISS controls, children with preterminal CRF, and those with ESRD. Bone age was delayed to a comparable degree in all 3 groups. The mean endogenous GH half-life in patients with ESRD was significantly higher than in ISS controls, while that in preterminal CRF patients was shorter than the value in ESRD patients but significantly higher than in ISS controls. A significant inverse linear correlation was observed between GFR and GH half-life. The mean number of detectable GH secretory bursts in ESRD children was greater than in ISS controls and in patients with preterminal CRF, while the interburst interval, the half-duration of the GH secretory burst, and the mean burst amplitude were not significantly different among groups. GH production rate (product of the mean mass of GH secreted per burst and the number of bursts/10 h) was significantly greater in ESRD patients than in CRF patients and tended to be higher than in ISS controls. Both mean and integrated plasma GH concentrations were significantly elevated in the ESRD group compared with the other 2 groups. However, plasma insulin-like growth factor 1 (IGF-1) levels did not vary among groups.

The authors state that the mechanism for increased GH secretion in uremia is unknown, but that the increased frequency of GH bursts is consistent with reduced somatostatinergic inhibitory tone. They theorize that such a decrease in tone could be due to either reduced hypothalamic or pituitary feedback action of IGF-1 or GH itself. IGF bioactivity is known to be reduced in uremic plasma due to the accumulation of binding proteins that are normally removed by renal filtration. This reduced bioactivity presumably leads to reduced feedback potency of IGF-1. Thus, the increased number of GH bursts, despite the increased GH concentrations, suggests tissue resistance to the actions of GH at the level of the hypothalamus. The authors speculate that the increased number of GH secretory bursts results from attenuated bioactive IGF-1 or GH feedback of the somatotropic axis and that this suggests an insensitivity to the action of GH in uremia.

Tönshoff B, et al. *Pediatr Res* 1995;37:86-93.

Editor's comment: *This is a very sophisticated analysis of the abnormalities in the GH/IGF-1 axis in children with CRF and ESRD. The authors point out that deconvolution analysis of spontaneous nocturnal GH secretion is exceedingly important to understanding the mechanism of GH secretion and its physiology and pathophysiology in a variety of disorders as well as in normal individuals.*

This particular study answers previous questions concerning the observation of normal IGF-1 and elevated GH levels in children with CRF and ESRD. It also aids in understanding the feedback mechanisms regarding GH and IGF-1 at the level of the hypothalamus and pituitary. However, what remains uncertain is why exogenous GH is so useful in stimulating linear growth in children with CRF. It would be interesting to perform studies similar to those reported in this paper on children before and after exogenous GH administration. Such studies might delineate how exogenous GH affects the GH/IGF-1 axis and its effect on tissue sensitivity to the actions of GH at the level of the hypothalamus.

William L. Clarke, MD

The Detection of Subtelomeric Chromosomal Rearrangements in Idiopathic Mental Retardation

About 3% of the population has an IQ <70; a cause is known in less than half. Chromosomal abnormalities identified by routine cytogenetic analyses account for an estimated 40% of severe mental retardation (MR) and an estimated 10% to 20% of mild MR. Subtle chromosomal defects that are not evident by routine testing could be responsible for a substantial portion of patients in whom a cause for idiopathic MR is not evident. Recent advances in molecular genetic techniques that allow detection of extremely small portions of chromosomes based on analysis of DNA polymorphisms (variable number of tandem repeats, or VNTRs) make it possible to detect such "cryptic" chromosomal defects.

Flint and colleagues used this approach to study 99 patients with idiopathic MR. Their attention focused on the subtelomeric portions of chromosomes because several known MR syndromes have been mapped to these regions and also because lesions in these regions might be repaired by telomeric repetitive DNA, masking the abnormalities from routine cytogenetic detection.

Using highly informative DNA markers that mapped to 28 chromosome tips (normal male karyotype has 48 short and long arm chromosome tips), they found cryptic rearrangements in 3 patients. One had a de novo deletion on the long arm of chromosome 13 and 2 others had de novo deletions of different sizes on the long arm of chromosome 22.

Thus, they found cryptic deletions in about 3% of the patients with MR whom they studied. The authors pointed out that 20 subtelomeric regions were not analyzed. If this was taken into account, together with the facts that the probes were not completely informative and in some instances mapped to regions not as close to the telomeres as they had wished, they estimated that the true frequency of cryptic subtelomeric deletions in MR is at least 6% and probably higher.

Flint J, et al. *Nat Genet* 1995;9:132-139.

Editor's comment: *What do mental and growth deficiency have in common? A lot more than sharing the term "deficiency." Both are multifactorial in their causation. The cause is not known in a high percentage of cases in both instances. They are associated with each other in many instances. Easily detectable chromosomal abnormalities are known to cause short stature and mental retardation, as in Down syndrome. Thus, it is not at all unreasonable to speculate that a substantial portion of "idiopathic" short stature might be caused by cryptic subtelomeric rearrangements as with MR. One has to assume that someone is already looking into this matter, and we look forward to the results.*

William A. Horton, MD

Normal Final Height After Treatment for Acute Lymphoblastic Leukemia Without Irradiation

Twenty-eight Dutch children (16 males, 12 females) with acute lymphoblastic leukemia (ALL) who did not have involvement of the central nervous system and who had achieved long-term remission after 1 course of treatment were followed. Median age at diagnosis was 4.4 years, with a range of 2.2 to 12.7 years. A variety of chemotherapeutic (CT) regimens were used, with a mean duration of treatment of 3.1 years and a range of 3.0 to 5.2 years. Treatment was administered between 1970 and 1986, with reevaluation 5 or more years after the completed therapy. Their heights at time of diagnosis were normal, declined significantly ($P<0.006$) during treatment, and then accelerated during the first 2 years after completion of therapy (Table 1).

Twenty-two children achieved final height. In 18, final height was greater than midparental target height, which was very encouraging. Body proportions, including sitting measurements, were normal. The therapeutic regimens employed for the management of ALL did intensify over the 16 years of treatment covered in this study, but no relationship between the treatment program and final height was demonstrable. The data support the concepts that: (1) CT influences growth negatively during treatment; (2) catch-up growth occurs; (3) final height is normal and sometimes even better than expected based on target height; and (4) body proportions are normal in those who attain normal final height. The authors did emphasize that further investigations are required to evaluate the influence of newer and more intensive CT regimens on linear growth and final height.

Table 1
Height Standard Deviation Scores
(n=28)

	Diagnosis	Completion of Treatment	Year 1	Year 2	Year 5
Median	-0.01	-0.17	0.11	0.24	0.29
Range	-2.1 to 2.8	-1.0 to 2.6	-0.9 to 2.8	-1.3 to 2.9	-1.3 to 2.7

Editor's comment: *Cranial irradiation unequivocally has adverse short- and long-term effects on the growth of children with ALL. The present data strongly suggest that CT alone does not permanently impair the growth of children who achieved a sustained remission after 1 course of treatment. The authors cite several other studies that support these findings. They also refer to an article by Sklar et al (*J Pediatr *1993;123: 59-64), who reported that CT alone led to a significant reduction in final height (-0.49 standard deviation score). Katz et al (*J Pediatr *1991;118:575-578), among others, observed no significant effect of CT alone on final height of children with ALL. The reason for the discrepant observations of the various groups is not obvious; however, the consensus of multiple studies is in accord with that of Holm et al.*

Holm K, et al. *Acta Paediatr* 1994;83:1287-1290.

Allen W. Root, MD

Delayed Adolescent Growth in Homozygous Sickle Cell Disease

This paper reports longitudinal observations of height in a Jamaican cohort of children with homozygous sickle cell (SS) disease, sickle cell hemoglobin C (SC) disease, and normal (AA) hemoglobin. The subjects were identified by neonatal screening of 100,000 consecutive deliveries at a major maternity hospital in Jamaica and included 315 children with SS disease, 201 with SC disease, and 250 AA controls (aged 11 to 18 years at the time of the study). All were followed prospectively every 3 months at the sickle cell clinic. The analysis was confined to postpubertal children with observations available to the age of 16 years. Parenthetically, 3 SS males with extreme retardation of sexual maturation who were growing normally for their bone age were excluded from the analysis as they were prepubertal and 29 of the SS group were excluded for various reasons. The final analysis included 44 SS patients (mean age, 17.9 ± 0.6 years; 21 males, 23 females), 44 SC individuals (17.3 ± 0.8 years), and 44 AA control children (17.9 ± 0.5 years).

Height was measured at 3-month intervals in SS and SC children and every 6 months in AA controls using a Harpenden stadiometer. In addition, sitting height was measured for SS and AA, but not SC children, at 6-month intervals using a sitting height table (Holtain Instruments). Tanner staging of the SS and AA subjects was assessed at 6-month intervals from the age of 8 years. The data was fitted by computer to the Preece-Baines model 1 (described in the article), which has been used previously to fit longitudinal height data. This particular mathematical model has the advantage of not requiring subjects' final height. It resolves complex growth curves into a variety of biologically meaningful parameters, including age of onset of the adolescent growth spurt and the age of peak height velocity. Multivariant analysis of variance was used to compare the growth parameters of the different groups. The significance levels were adjusted using Bonferroni's method of correction.

The data demonstrate that SS patients have a 1.4-year delay in mean age of initiation of the adolescent growth spurt, a 1.6-year delay in mean age at peak height velocity, and a lower height velocity at the time of the onset of the growth spurt compared with AA controls. The first pubertal changes (Tanner stage 2) occurred at 12.8 ± 1.6 years in SS males and 12.0 ± 1.8 years in SS females, compared with 11.1 ± 1.2 years in AA males and 10.1 ± 1.2 years in AA females. Adjusting for the age of onset of the growth spurt or peak height velocity reduced the genotype difference from 1.8 to 1.2 years. The delay in onset of puberty in SS patients compared with AA controls correlated with their delay in peak height velocity. The age of menarche in SS girls was significantly later than in AA girls (15.4 ± 1.3 vs 13.1 ± 1.3 years; $P<0.001$). After adjusting for the delay in the adolescent growth spurt, the genotype difference in age of menarche was no longer significant.

The authors suggest that error could be introduced into their data by studying children as early as 1 year of age, inaccuracy in measuring young children, measurement error due to observer variation over the 18-year span of the study, and technical variation arising from changing hairstyles. They further suggest that the etiology of the delays observed may be multifactorial, with contributions from abnormal endocrine function (including hypogonadism), suboptimal nutrition, and increases in metabolism as the result of a high rate of erythropoiesis. Interestingly, these changes do not affect the final heights of these children.

Singhal A, et al. *Arch Dis Child* 1994;71:404-408.

Editor's comment: *This is a very carefully performed study. The authors are to be congratulated for identifying individuals in the neonatal period and following them through their adolescent growth spurt. It is unfortunate, however, that more information either was not collected or provided regarding the etiology of the retarded growth or the delay in onset of puberty in these children. The pattern observed seems consistent with constitutional delay of growth and adolescence. It is unclear how abnormal endocrine function, suboptimal nutrition, or increased metabolism could have a transient effect on the onset of puberty and yet not affect final height. Interestingly, there is no mention of rates of infection, number of hospitalizations, or numbers of crises, all of which may have temporarily affected growth in enough children to produce the observed delay in growth. Since the SS children achieve a normal final height, one might question the desirability of continuing research into the etiology of their delay. However, these individuals appear to provide a model of constitutional delay of growth and adolescence that might prove useful in better understanding the physiology of the timing of pubertal events in healthy children.*

William L. Clarke, MD

Gene Therapy for Familial Hypercholesterolemia

In theory, diseases caused by genetic deficiency can be treated by the introduction and expression of a normal gene into the affected tissue. Because of the possibility of a noninvasive and accurate monitoring method for familial hypercholesterolemia (FH), and based on previous promising results of gene therapy in animal models (Chowdhury et al[1]), Grossman et al[2] recently reported the first successful ex vivo gene therapy treatment for FH in a human, specifically, in a 29-year-old woman.

FH is an autosomal dominant disorder caused by a deficiency of low density lipoprotein (LDL) receptors. Patients with FH have very high blood levels of cholesterol that deposits in the coronary arteries and leads to premature coronary artery disease (Brown and Goldstein[3]). The homozygous form of FH is a lethal disorder. It is very hard to treat; however, the progress and response to treatment can be easily monitored by measuring serum lipid profiles.

The protocol reported by Grossman and colleagues[2] was as follows: a partial liver resection was performed on the patient (15% of the total mass) and the liver section was perfused with collagenase to obtain hepatocytes, which were then cultured. The cells were exposed to recombinant retroviruses that had a new gene recombined into their DNA that contained the LDL receptor. The genetically-corrected hepatocytes were harvested and infused back into the patient via the inferior mesenteric vein, leading to their deposit in the liver. The patient's serum lipid profile was measured before and after treatment. Two weeks after the procedure, the ratio of LDL to high density lipoprotein (HDL) was noted to drop from 10:13 to 5:8. The patient remained stable for 18 months without further complications. The authors concluded that hepatic reconstitution of LDL receptor expression is sufficient for metabolic correction.

1. Chowdhury JR, et al. Long-term improvement of hypercholesterolemia after ex vivo gene therapy in LDLR deficient rabbits. *Science* 1991;254:1802-1805.
2. Grossman M, et al. Successful ex vivo gene therapy directed to liver in a patient with a familial hypercholesterolaemia. *Nat Genet* 1994;6:325-341.
3. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986;232:34-37.

Editor's comment: *The use of gene therapy such as that reported here is encouraging. In FH, the liver is an easy organ to target. Successful gene therapy in disorders primarily involving a specific organ may be easier to achieve than gene therapy for disorders that affect many systems. The possibility of removing cells from the affected individual, treating them, and then reinserting them avoids the possibility of rejection and further complications related to immunosuppression. Unfortunately, the same type of approach cannot be easily used in cystic fibrosis.*

Judith G. Hall, MD

Gene Therapy for Cystic Fibrosis

Cystic fibrosis (CF) is a common autosomal recessive disorder. It is characterized by gastrointestinal and respiratory symptoms. The pulmonary complications of CF include mucus plugging and chronic bacterial infections. Ninety percent of CF patients die of respiratory complications. Because the high mortality of CF is related to respiratory symptoms, the lungs have been the logical target for gene therapy, as reported by Cutting.[1]

CF is caused by a mutation of the CF transmembrane conductance regulator (*CFTR*) gene on chromosome 7. The *CFTR* gene codes for a transmembrane channel on the surface of the epithelial cells that affects electrolyte transport and balance. *CFTR* mutations result in the mislocalization of the protein or in reduced function at the membrane that leads to an abnormal electrolyte exchange and, consequently, very thick pulmonary and intestinal secretions.

Earlier work on gene therapy for CF was directed at the respiratory epithelial cells of mice. Human epithelial airway tissue is one site of the expression of the disorder. However, targeting respiratory epithelial cells has been difficult, mainly because the epithelial tissue is composed of a number of different cells at different stages of differentiation, and it is unclear which are the cells that express the defective *CFTR* gene.

Recently, Crystal et al[2] reported their results of gene therapy with a recombinant adenovirus vector (Ad*CFTR*) containing the human *CFTR* cDNA, administered to the respiratory tracts of 4 individuals with CF. All individuals had baseline evaluation of the respiratory epithelium before and after the administration of Ad*CFTR*. The Ad*CFTR* was then inhaled by the patients. The number of "corrected cells" was difficult to assess, but the epithelial cells did express the corrected *CFTR*. The authors concluded that it is feasible to use an adenovirus vector to introduce the gene and to achieve expression of the normal human *CFTR* in the epithelial tissue in a living patient. They point out, however, that their study does not establish whether this therapy will be successful in treating the common respiratory symptoms of CF or whether incorporation will be stable for long periods. No change in respiratory function was noted.

1. Cutting GR. Two steps closer to gene therapy for cystic fibrosis. *Nat Genet* 1992;2:4-5.
2. Crystal RG, et al. Administration of an adenovirus containing the human *CFTR* cDNA to the respiratory tract of individuals with cystic fibrosis. *Nat Genet* 1994;8:42-51.

Editor's comment: *Gene therapy for CF has proven "tricky." Animal studies have been encouraging in that the gene can be incorporated, so the next step was to try gene therapy in humans. However, since CF involves the lung, pancreas, and other organs in humans, the incorporation of the gene is harder to assess. It is unclear whether the respiratory symptoms accessible for monitoring improve after gene therapy, and it may be that the improvement is only temporary. The dosage of gene therapy has been problematic as well. Perhaps the "trick" is to concentrate on targeting only one organ for treatment and involve the stem cells. The question then becomes what organ or which tissue in that one organ should be targeted?*

Judith G. Hall, MD

Body Composition and Spontaneous GH Secretion in Normal Short Stature Children

In adults, the effect of obesity in suppressing growth hormone (GH) release has been well studied over 30 years. In children, only a few studies to determine the effect of obesity on GH release have been published. Abdenur et al attempt to fill that void in the study reported in this article.

Fifteen pubertal and 22 prepubertal short normal children were studied in relation to auxologic parameters, with emphasis on the measurements of body fat (BF) composition and the relationship of BF with spontaneous GH secretion (SGHS) over 12 hours at night.

A significant negative correlation between the degree of adiposity and mean SGHS was reported. A strong negative correlation was demonstrated with %BF as determined by bioelectrical impedance and BF mass index (BFMI), which is calculated as BF in kilograms/height in meters squared (Figure 1). Females required greater adiposity levels than males to decrease SGHS in pubertal subjects. Correlation between SGHS and BF was best with the mean pulse amplitude in pubertal subjects and the number of pulses and the sum of pulse amplitudes in prepubertal subjects.

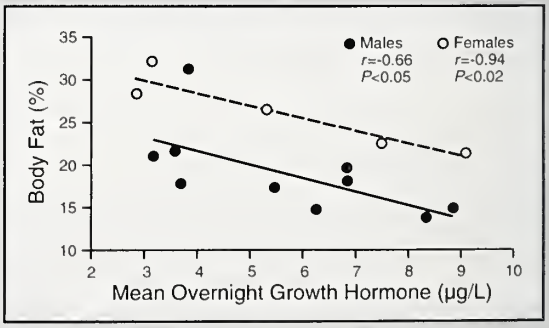
The authors conclude that in normal short-statured children, body composition greatly influences SGHS. Consequently, SGHS levels that appear low may actually be normal for a short child with mildly increased BF, and SGHS values that appear normal may be abnormally low for a lean individual who would be expected to have high SGHS levels because of leanness. This is an extremely important concept for a patient being evaluated for possible relative alterations in GH secretion. These results suggest also that normal values for SGHS must take into account not only pubertal status but also gender and body composition. The use of a mathematical formula that considers SGHS and IGF-1 values has been proposed (Oerter KE et al, *J Clin Endocrinol Metab* 1992;75:1413-1420). However, with a sufficient number of male and female patients, a more practical approach would be the use of confidence intervals to define normal values of SGHS according to body composition and gender.

Abdenur JE, et al. *J Clin Endocrinol Metab* 1994;78:277-282.

Editor's comment: *The authors are to be commended on performing and presenting a much needed study pertaining to the correlations of SGHS and various measurements of BF. Space did not permit an elaboration of the different important ways that BF was assessed.* GGH *readers are encouraged to read this article in its entirety, particularly to attain better comprehension of the various ways that BF can be measured and what those parameters really mean and reflect.*

Robert M. Blizzard, MD

Figure 1
% Body Fat and Mean SGHS: Correlation Analysis



Correlation between the %BF and mean overnight GH secretion in pubertal patients.

Growth of Short Normal Children in Puberty Treated for 3 Years With Growth Hormone (GH) Alone or in Association With Gonadotropin-Releasing Hormone Agonist (GnRHa)

GH at 0.1 IU/kg/d, 6 days per week (≈0.2 mg/kg/wk) was given to 30 early pubertal short normal subjects for 3 years; 16 males, aged 14.4 ± 0.8 yrs; 14 females, aged 12.2 ± 1.2 yrs, all at Tanner stage 2 or 3, with slow pubertal growth (4.2 ± 1.2 cm/yr), a mean BA delay of 2 years, and no detected GH deficiency or other cause for short stature. Their mean birth length was 48.6 to 49.5 cm at term; the mean of midparental heights was -0.6 to -0.8 SD below the mean of the general adult population. They were randomized in two groups: group A received GH alone; group B received GnRHa for 2 years plus daily GH injections, and on the third year GH alone.

The annual growth velocity (GV) increased during the first year in both groups and sexes, the increase being significant ($P<0.01$) in group A only. The patients of group A kept an improved GV in the 2nd year, then returned to pretreatment GV in the 3rd year, while completing their sexual development and bone maturation. Their height, expressed as SDS for bone age, improved in the first two years but decreased thereafter. Group B patients returned to pretreatment GV in the 2nd year, and had no significant improvement when treated with GH alone during the 3rd year of the study. They had no significant progress of height for age at any time. Their bone maturation, slow when on the GnRHa accelerated when sexual development resumed.

At the end of the 3 years, height expressed as SDS for age improved in group A from -2.5 ± 0.6 to -1.5 ± 0.4 SD in males ($P<0.05$) and from -2.8 ± 0.5 to -2.1 ± 0.9 SD in females (NS). Expressed as SDS for bone age, mean height slightly improved in males (NS) but not in females. In both groups and sexes, the mean predicted height according to Bayley and Pinneau was only slightly increased at the end of 3 years on GH, with a gain of 2 to 5 cm on the average. There was a wide interindividual variability in these results within each group. Pretreatment characteristics of the patients did not account for individual differences. Annual measurement of plasma insulin-like growth factor 1 (IGF-1) showed different degrees of increase, not correlated with any parameter of the patients' growth.

The authors reached 2 conclusions. First, inhibiting sexual development in short early pubertal subjects has no advantage. This was previously demonstrated with GnRHa alone (see *GGH* 1993;9[4]:13), and now is confirmed for GnRHa plus GH. Second, GH alone, at the dose used, can accelerate for 2 years the growth of such slow-growing normal short adolescents and slightly improve their predicted height in relation to the result of the first year of treatment, but the expected results cannot be overestimated or considered as an indication for any routine use of GH in endocrinologically normal and constitutionally short pubertal individuals.

Job JC, et al. *Horm. Res* 1994;41:177-184.

Editor's comment: *The contents of this report will discourage only the most desperately short children from trying to achieve normal height by using GnRHa plus GH.*

Robert M. Blizzard, MD

Catch-up Growth, Persisting Short Stature, and Adult Height of Children Born Small for Gestational Age

Two recent papers give large-scale data on the natural history of statural growth in children born with intrauterine growth retardation (IUGR), defined as -2 standard deviations below the mean reference values of Usher and MacLean.

One is a longitudinal retrospective study[1] of a cohort of 3,650 healthy individuals born at full term in Sweden who have reached their adult height when arriving in the final grade of school. The growth data of those who were born with weight and length in the normal range were used as reference values. The values in IUGR children (n=198) were calculated separately for those born with a subnormal length (n=141) or a subnormal weight (n=111), and for those born both short and light (n=54).

A spontaneous catch-up growth occurred before age 2 years in 87% of the total IUGR group. The 13% whose height remained ≤-2 SD at age 2 years were all from the short-at-birth group. They remained in the subnormal range of height throughout childhood. Their puberty started at a normal time, somewhat early. Their mean final height was -1.7 SD. It is to be noted that midparental height was approximately -1 SD in this non–catch-up group.

The other study[2] reports the final heights of 47 healthy subjects (23 males, 24 females) followed in pediatric endocrine clinics for severe height retardation of prenatal onset. Their mean birth length at term was <-2 SD. They were referred after the age of 4 years for persistence of a height deficit of >2 SD. Patients with malformation syndromes and those with subnormal GH responses to usual stimulation tests were not included in the series studied. Puberty started late for chronologic age but early for bone age: in males at 14.2 ± 0.8 years with bone age of 11.9 ± 0.7 years and a mean height of 139.2 ± 4.5 cm; in females at 12.4 ± 0.7 years with a bone age of 10.1 ± 0.7 years and a mean height of 130.9 ± 6.2 cm. The mean pubertal growth was 23.0 ± 4.0 cm in boys and 15.5 ± 4.6 cm in girls.

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The 23 males thus reached an adult height of 161.9 ± 8.0 cm, and the 24 females 147.6 ± 7.2 cm. Bone age before puberty or at the onset of puberty was not a valuable individual predictor of final height in these IUGR children. The adult heights correlated significantly with birth length (r=0.45), with height at age 2 years (r=0.50) and closely with height at the onset of puberty (r=0.81), but not with birth weight or with midparental heights.

1. Albertsson-Wikland K, Karlberg J. *Acta Paediatr Scand* 1994; 399(suppl):64-70.
2. Chaussain JL, et al. *Acta Paediatr Scand* 1994;399(suppl): 72-73.

Editor's comment: *Very few data had been previously reported regarding the statural growth of small-for-date newborns beyond childhood and were somewhat discrepant, probably since they were based on either birth length or weight. This situation was reflected in a study previously abstracted in* GGH. *In spite of their very different protocols, the two studies summarized here (the second one having been abstracted in* GGH *previously [1993;9(4):10] agree on the same main facts; (1) in long-term growth studies, birth length, not birth weight, is the criterion to take into consideration for definition of IUGR; (2) in more than 85% of infants born short at term, a spontaneous catch-up growth occurs before the age of 2 years; and (3) those IUGR children who remain short at the onset of puberty do not experience pubertal catch-up growth. These points seem important for the design of clinical trials using GH or growth-related peptides in IUGR children. The data collected will be useful as historical references when the final heights of IUGR children presently involved in trials with GH will be known.*

Jean-Claude Job, MD

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc. The information in this publication reflects the views of the editors and/or contributors and not necessarily those of the grantor or the publisher.

Published by:
Gardiner-Caldwell
SynerMed
405 Trimmer Road
PO Box 458
Califon, NJ 07830

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc.

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GROWTH
Genetics & Hormones
Vol. 11 No. 3 September 1995

Letter From the Editor

To Our Readers:

The December 1995 issue (Vol. 11, No. 4) of *GROWTH, Genetics, & Hormones (GGH)* will be an index issue. This cumulative index will list all of the lead articles and each of the literature abstracts published during the last 11 years. The special index issue is in response to multiple requests for such a comprehensive listing—a testament to *GGH*'s extensive use as a reference source. Watch for the December issue—it will be of considerable value to you.

If *GGH* has been a significant resource for you, please be encouraged to write to me, in care of SynerMed, expressing your appreciation to Genentech, Inc. for its support in funding this publication over the years.

For the Editorial Board,

Robert M. Blizzard, MD
Chairman, *GGH* Editorial Board

The Neuroendocrinology of Puberty

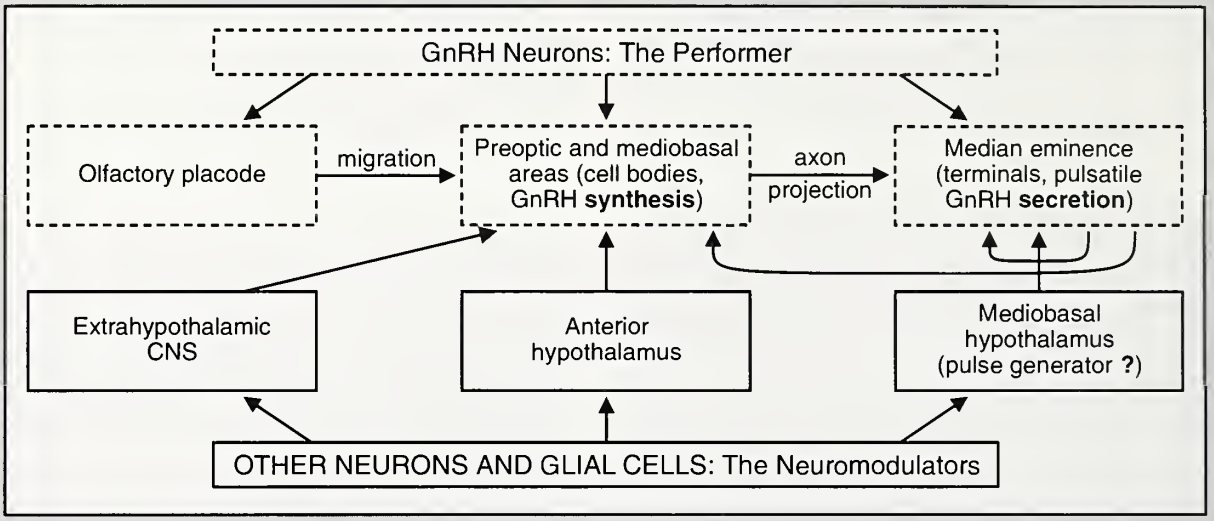
Jean-Pierre Bourguignon, MD, PhD
Professor of Pediatrics, University of Liège, Liège, Belgium

Central nervous system (CNS) control of the reproductive hormonal axis was well recognized 75 years ago by clinicians caring for patients with hypothalamic lesions and associated disorders of puberty. During the past 20 years, a vast body of information has accumulated about the neuroendocrinology of the reproductive axis. However, the complex changes that combine to trigger the onset of puberty remain a puzzle that is not yet solved. It is hoped that the facts and presumptions presented in this review will provide a framework for the further development and testing of hypotheses to help unravel the mysteries of puberty. The functional, anatomic, and biochemical aspects of the neuroendocrinology of pubertal development will be discussed sequentially, as well as how these intrinsic hypothalamic processes are modulated by gonadal steroids and extrahypothalamic signals to influence the onset of puberty.

FUNCTIONAL MANIFESTATIONS OF THE NEUROENDOCRINOLOGY OF PUBERTY

Direct gonadotropin hormone-releasing hormone (GnRH) measurements in vitro from rat hypothalami and in vivo from the hypophysealportal circulation of the rhesus monkey support the concept that the onset of puberty is marked by an increase in frequency and amplitude of GnRH secretion.[1,2] In humans, the same conclusion is supported indirectly by sensitive gonadotropin measurements, which have been used to track hypothalamic GnRH secretion in the peripheral circulation. Serum luteinizing hormone (LH) concentrations reflect the secretion of detectable pulses in prepubertal children, the frequency of which increases by 2-fold, particularly at night, during the late prepubertal period.[3-5] In addition, LH pulse amplitude increases as a result of changes in hypothalamic function and pituitary sensitivity to GnRH. As shown by Knobil and coworkers in primates, the increased frequency of intermittent stimulation of the pituitary gland by

Figure 1
Anatomic Basis of the Neuroendocrinology of Puberty



GnRH is critical for the appropriate increase in gonadotropin secretion at the onset of puberty.[6,7] Others have confirmed that the receptivity of the gonadotropes to GnRH is frequency-modulated. Thus, studies on the hypothalamic mechanism in the onset of puberty have focused on the control of pulsatile GnRH secretion and its ontogeny.

THE ANATOMIC BASIS OF THE NEUROENDOCRINOLOGY OF PUBERTY

The hypothalamic involvement in the neuroendocrinology of puberty can be viewed as a 2-compartment model consisting of the GnRH neurons and the neuromodulators released from other neurons and/or glial cells (Figure 1). The GnRH neurons represent the performer, the ultimate structure where most neural factors converge to regulate pituitary-gonadal activity, although some factors might act at the pituitary to moderate or to prime the action of GnRH. GnRH neurons have a fascinating developmental history. They arise outside the brain in the olfactory placode and subsequently migrate during fetal life to the preoptic area in rodents—and even farther in primates—to the anterior hypothalamus.[8] In humans and primates this process takes place during the first trimester of pregnancy, whereas it occurs during the second half of gestation in rodents. This migratory process is complex and likely depends on several factors, one of which has homology with the neural cell adhesion molecule, a member of a family of adhesion factors interacting to attach neurons to axons to defined brain structures during migration or growth processes; it is encoded by a gene on the short arm of the X chromosome (Xp22.3). When this *Kal-1* gene is deleted or mutated, there is a defect in the migration of both GnRH and olfactory neurons, which gives rise to the hypogonadotropic hypogonadism and anosmia of Kallmann syndrome.[9] From their normal position in the anterior hypothalamus, GnRH cell bodies project a majority of their axons caudally to the median eminence, where they terminate on the hypophyseal portal vessels. GnRH is initially synthesized as pro-GnRH (a precursor), which is prominently present in the GnRH cell bodies. In the male rat, GnRH mRNA and the content of pro-GnRH increase markedly at 20 to 24 days of age, immediately preceding the onset of puberty.[10] During axonal transport, the GnRH precursor is processed into the mature decapeptide form, which is primarily located and stored in the arcuate nucleus-median eminence area.

While there is general agreement that GnRH pulsatility is crucial to stimulate physiologic gonadotropin secretion, there is no consensus as to what constitutes the "pulse generator." An interesting model is provided by GnRH neurons immortalized through targeted tumorigenesis using an oncogene coexpressed with the GnRH gene.[11,12] The intermittent secretion by immortalized GnRH cells in vivo provides evidence that pulsatility could be an intrinsic property of the GnRH neurons.[11,12] The requirement for synchronous secretion to yield the GnRH pulses measured in vitro and in vivo suggests that there is cross talk between GnRH neurons. The concept of such a mechanism is further supported by ultrastructure data revealing interconnections between GnRH neurons in vitro and in vivo. The potential role of GnRH itself as the coordinator of

synchronous activity of a population of GnRH neurons is supported by experiments demonstrating inhibitory autofeedback.[13,14]

Alternatively, it is possible that a pulse generator distinct from GnRH neurons exists. There is evidence that there is in the rat mediobasal hypothalamus a pacemaker distinct from the GnRH neuron, since LH and GnRH pulsatility continue following disconnection of GnRH axons from their cell bodies in vivo[15,16] and in vitro.[2] This hypothesis is consistent with electrophysiologic recordings in vivo. In primates, Knobil and colleagues have localized the GnRH pulse generator electrophysiologically in the mediobasal hypothalamus.[6,7] However, the nature of the pacemaker has not been elucidated.

The neuromodulators (Figure 1, lower section) signaling to GnRH neurons may originate from neurons or glial cells in extrahypothalamic areas of the CNS, as well as in the anterior and mediobasal hypothalamus, and may exert their effect by impinging on GnRH neuronal cell bodies in the preoptic area and, presynaptically, on GnRH nerve terminals in the arcuate nucleus-median eminence. Anatomic changes in these interactions during development may involve particular processes such as synaptic plasticity or programed cell death. The role played by glial cells can be pivotal through the production of potently active peptides such as transforming growth factor-α (TGF-α)[17] or enzymes controlling the biosynthesis and degradation of neuropeptides or neurotransmitters. As an example, we showed very recently that glutaminase, an enzyme produced by astroglial cells and involved in the biosynthesis of glutamate, played a critical role in pulsatile GnRH secretion.[18] In summary, the anatomic basis for the regulation of GnRH synthesis and secretion is complex and includes an array of neuronal-glial elements in the hypothalamus.

THE BIOCHEMICAL MECHANISMS INVOLVED IN THE NEUROENDOCRINOLOGY OF PUBERTY

Conceptually, puberty may result from either *decreasing inhibition or increasing facilitation* of GnRH secretion. Progressive dissipation of inhibition has been considered as the most likely hypothesis since the hypothalamic pulse generator is active during human fetal life, subsequently becomes restrained, and then reactivates.[19] However, in the rat, early activation of GnRH and, consequently, LH secretion preceding the restrained, or so-called juvenile pause, period has not been observed so far, which is in contrast to the observed early activity in primates and humans.

Numerous neuromodulators are known to affect GnRH secretion as neurotransmitters or neuropeptides.[20] Some directly inhibit and others directly stimulate GnRH secretion. A number of neurotransmitters and peptides are dual regulators, since they show both inhibitory and facilitatory effects in different experimental conditions (Figure 2). Specific anatomic sites probably come into play, as a single neuromodulator may exhibit different effects in the preoptic area than in the mediobasal hypothalamus. Puberty can be viewed as resulting from either a change in the activity of neuromodulators, such as a reduction of inhibition or an increase of stimulation, or, alternatively, a change in sensitivity of GnRH neurons to the effect of neuromodulators—or even as a result of both mechanisms (Figure 2). It is important to remember that most data have been

Figure 2
Possible Biochemical Regulators and Mechanisms Involved in the Neuroendocrinology of Puberty

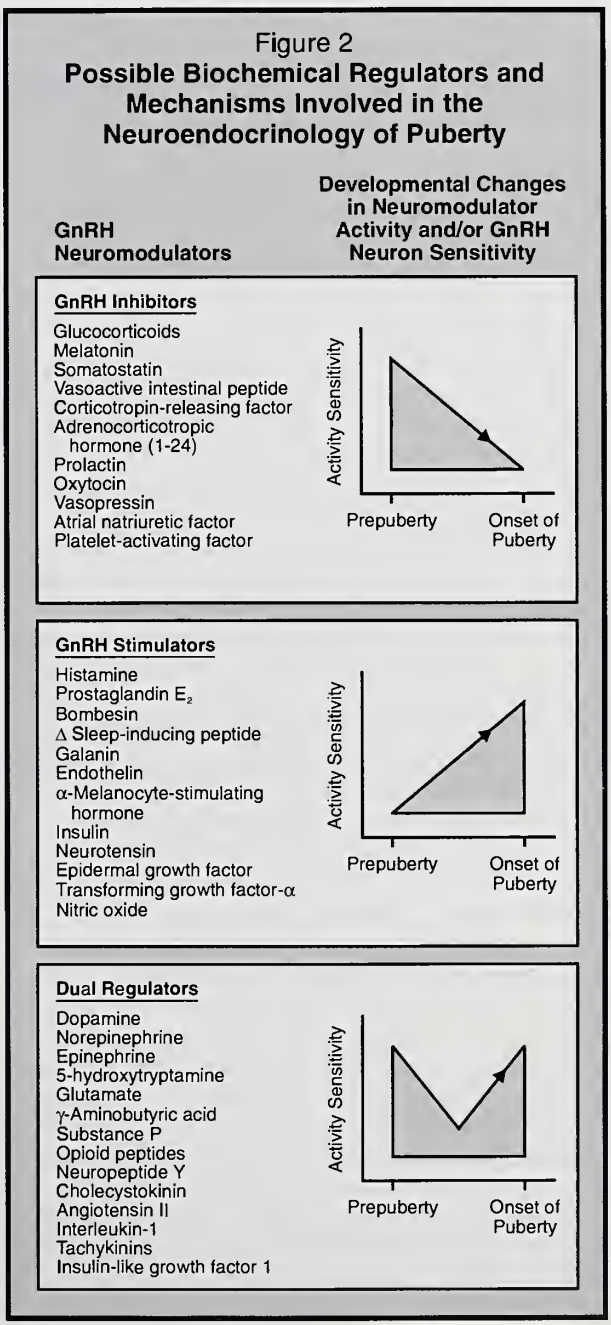
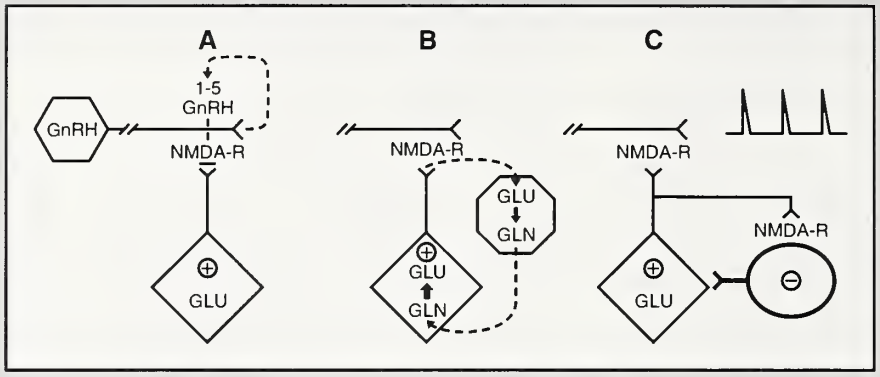


Figure 3



Three different mechanisms could account for loop circuits controlling the interval between episodes of stimulation of GnRH secretion by glutamatergic neurons in the male rat hypothalamus. At onset of puberty, the increase in frequency of pulsatile gonadotropin hormone-releasing hormone (GnRH) release may involve (A) reduction in potency of competitive antagonism at N-methyl-D-aspartate receptors (NMDA-R) by 1-5GnRH, a physiologic degradation product of GnRH; (B) increased rate of restoration of the releasable pool of glutamate (GLU) by glutaminase, the enzyme controlling GLU biosynthesis from glutamine (GLN); and (C) disappearance of inhibitory GABAergic interneurons activated through NMDA receptors.

obtained in the rat, and that the effects of neuromodulators may be different in rodents than in primates or humans.

Since the GnRH neurons attain full functional capacity early in fetal life, the prepubertal pause of secretory activity of those neurons is likely to involve a superimposed brake driven by distinct neurons, presumably in the pulse generator. Our work in the male rat has highlighted the possible role of glutamatergic neurons in such a mechanism (Figure 3). The developed concept relies on intermittent presynaptic stimulation of GnRH axons by glutamatergic neurons through facilitatory N-methyl-D-aspartate receptors (NMDA-R) so that GnRH secretory pulses are generated. Three different mechanisms theoretically can account for loop circuits controlling the interval between episodes of stimulation of GnRH secretion by glutamatergic neurons (Figure 3). The 1-5GnRH fragment, a physiologic breakdown product of the secreted decapeptide, can act as a competitive antagonist at NMDA receptors and so contribute to the inhibitory autofeedback of GnRH (Figure 3A).[14] If physiologically relevant, the onset of puberty could involve reduction in potency of, or sensitivity to, that autofeedback.[13] A second possible mechanism is one based on the increase in glutaminase activity that is observed after the onset of puberty (Figure 3B).[18] Glutaminase controls the biosynthesis of glutamate from glutamine, which is synthesized by astroglial cells from glutamate after reuptake of the excitatory amino acid from the synaptic cleft. It is not known whether the age-related increase in glutaminase activity is causal or consequential to the presumably increased frequency of glutamate secretory discharges. A third possible mechanism is one based on neurotransmitter inhibition of GnRH pulsatility. This inhibitory effect, which is observable only before the onset of puberty, involves glutamate and NMDA receptors, thus pointing to their dual role in the regulation of GnRH secretion (Figure 3C).[21] Such inhibition is conceivably mediated through interneurons expressing NMDA receptors and inhibitory to the pulse generator. Based on recent observations in the monkey, it can be postulated that those interneurons are GABAergic.[22] At the onset of puberty, disclosure of the facilitatory effect of glutamate might result from the marked reduction in activity of those inhibitory interneurons. We think that the latter mechanism is likely to play a major role at the pubertal onset, while the other mechanisms, which are still working following the onset of puberty, may contribute to the regulation of the frequency of pulsatile GnRH secretion in the adult hypothalamus.

THE GONADAL ROLE IN THE NEUROENDOCRINOLOGY OF PUBERTAL DEVELOPMENT

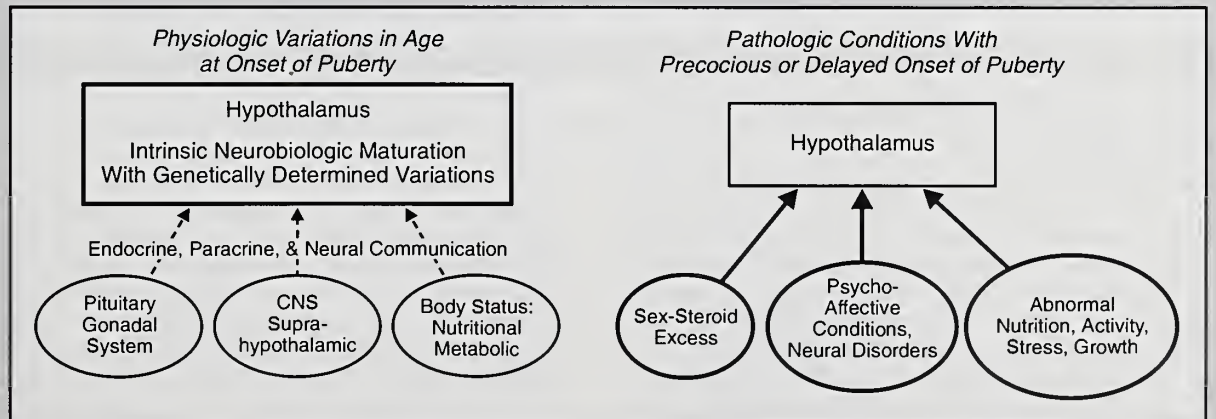
The role of sex steroids in puberty has been studied extensively in pursuit of clarifying the gonadostat hypothesis.[23] Since sex steroids undoubtedly influence the neuroendocrine system, it is particularly difficult to delineate whether neuroendocrine manifestations of puberty are primary maturational events in the hypothalamus that account for increased pituitary-gonadal activity or secondary events resulting from increased sex steroid secretion. Currently, the neuroendocrine mechanism of puberty is believed to involve 2 components with respect to the role of the gonads. The gonadal-dependent component is illustrated by the qualitative effects of sex steroids on the activity of opioid peptides, neuropeptide Y, norepinephrine, and dopamine. These neurotransmitters exhibit opposite actions on LH or GnRH secretion in the absence or in the presence of sex steroids.[20] The gonadal-independent component of

neuroendocrine maturation is illustrated by the data obtained in orchidectomized monkeys[24] and in agonadal patients,[25] who show developmental changes in gonadotropin secretion similar to normal subjects. Gonadotropin secretion is greater during infancy and at the time of adolescence than during the juvenile pause. This gonadal-independent component of maturation is quantitatively dependent on sex steroids since gonadotropin secretion during infancy and adolescence is greater in agonadal patients or animals than in normal subjects. It is critical to consider whether any change in the neuroendocrine control of GnRH-LH secretion at puberty is causal or consequential to increased sex steroid secretion. In the rat, we found that the developmental pattern of changes in NMDA receptor–mediated control of GnRH secretion was similar in both intact and orchidectomized animals, thus indicating that glutamate effects were qualitatively independent of sex steroids.[26] There was also a quantitative effect of sex steroids irrespective of age, since prepubertal and adult orchidectomized animals showed evidence of increased glutamatergic stimulation of GnRH secretion compared with intact animals.[26] Therefore, glutamate effects on GnRH secretion appear to involve an age-related, gonadal-independent component, as well as a gonadal-dependent component.

INTRINSIC HYPOTHALAMIC MATURATION AND EXTRAHYPOTHALAMIC SIGNALS: THEIR INFLUENCE IN STIMULATING PUBERTAL DEVELOPMENT

In addition to gonadal factors, the hypothalamus is directly under the influence of the suprahypothalamic CNS and affected by the nutritional and metabolic state of the body (Figure 4). Signals from those systems may be communicated to the hypothalamus along paracrine, endocrine, or neural pathways. That changes in these impact on the mature hypothalamus is well established, but the signals remain to be clearly identified. However, it is not clear that the same pathophysiologic mechanisms that modulate the adult reproductive axis play any role in the neuroendocrine mechanism of the onset of puberty. This is a most difficult question to study because there are major species and sex differences.[27] For instance, the light/darkness cycle plays a critical role in seasonal breeders, while little or no such effect exists in humans. In humans, the impact of systems external to the hypothalamus seems to be rather low in physiologic conditions, compared with most animal models. Thus, under optimal conditions, physiologic variations in the age at onset of puberty, for example, could be related to genetic variations in the process of intrinsic neurobiologic maturation in the hypothalamus. In contrast, there is evidence that acute as well as chronic deviations from normal conditions of nutrition, activity, stress, and/or growth result in striking effects on pulsatile LH secretion and puberty. Abolition of LH pulsatility occurs during periods of fasting or strenuous physical activity.[28,29] Delayed puberty occurs in patients with isolated growth hormone (GH) deficiency.[30] Early puberty is seen following catch-up growth in adopted children recovering from nutritional and psychoaffective deprivation.[31] Sex steroid excess or premature increase, such as that seen in untreated congenital adrenal hyperplasia or gonadotropin-independent sexual precocity, also may result in secondary precocious hypothalamic maturation. While these extrahypothalamic signals may play a prominent role in abnormally precocious or delayed puberty, they probably play a much less important role in normal physiologic maturation.

Figure 4



Relative roles of intrahypothalamic maturation and extrahypothalamic signals in physiologic and pathologic variations in age at onset of puberty.

SUMMARY

In summary, the neuroendocrine mechanisms involved in triggering the onset of puberty produce an acceleration of pulsatile GnRH secretion resulting from complex anatomic interactions among GnRH neurons, other regulatory neurons, and glial cells. Many different neuromodulators may play inhibitory, facilitatory, or dual inhibitory-facilitatory roles, with glutamate and GABA currently leading this list. While the influence of extrahypothalamic factors may be minor in physiologic conditions, their effects could be prominent in disorders of puberty. Much remains to be delineated. However, the building blocks for further investigation are now in place.

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Letter From the Editor

Dear Colleague:

Genetics indeed is a very broad area of interest. The abstracts submitted by Drs. Judith Hall and William Horton for this issue of *GROWTH, Genetics, & Hormones (GGH)* particularly emphasize to me that a disease is usually not just a disease, but a symptom complex that may have multiple etiologies. For that reason, the following abstracts have been grouped together. Such a grouping emphasizes the similarities and dissimilarities of various chondrodysplasias in relation to their phenotypes and in relation to their genetic and/or biochemical cause. Hopefully, you will enjoy reading them as a group because, to use an analogy, you can better comprehend the trees by seeing the forest and better comprehend the forest by seeing the trees. Perhaps we can better enjoy and learn by studying the substantive relationships within and among these abstracts. We will attempt to do more grouping of this type in *GGH* in the future. We hope you approve.

Robert M. Blizzard, MD
Chairman, *GGH* Editorial Board

Abstracts From the Literature

A Constitutively Active Mutant PTH-PTHrP Receptor in Jansen-Type Metaphyseal Chondrodysplasia

Jansen-type metaphyseal chondrodysplasia (JMC) is a rare but distinctive autosomal dominant skeletal dysplasia. Patients have severely short limbs, prominent skull bones, and enlarged joints that become severely deformed in adulthood. Skeletal radiographs show demineralization and rachitic-like changes in the metaphyses in infancy and severe widening and irregularities of the metaphyses in childhood. These radiographic changes and the hypercalcemia and hypophosphatemia that these patients display suggest a link to hyperparathyroidism. However, parathyroid gland histology and blood parathyroid hormone (PTH) and PTH-related protein (PTHrP) levels are unremarkable.

There are other types of metaphyseal chondrodysplasia that appear clinically similar to JMC, such as the Schmidt type; however, these 2 now have been demonstrated to be of totally different genetic origins. The Schmidt type results from heterozygous mutations of the type X collagen gene, and these are thought to diminish the amount of this protein in the growth plate. JMC, as stated in the current study, is reported to be attributable to a receptor defect that changes a strictly conserved histidine residue at position 223 in the receptor protein's first intracellular loop to arginine.

Persistent suspicion of a disturbance in the PTH-PTHrP-calcium axis prompted the investigation of the receptor for this

axis in a patient with JMC. The investigators determined that a heterozygous mutation prompted the arginine substitution at position 223. This histidine is highly conserved among members of the G protein-coupled transmembrane receptor family to which the PTH-PTHrP receptor belongs. Findings provide evidence that the recently isolated receptor is the major mediator of PTH and PTHrP action. The authors believe that a paracrine-autocrine role for PTHrP may exist, as PTHrP and the PTH-PTHrP receptor are both expressed in adjacent cells within the metaphyseal growth plate.

Intriguingly, the authors present findings that support the hypothesis that "activating" receptor mutations may cause abnormal formation of endochondral bone as well as abnormalities in mineral anion homeostasis.

One of the fascinating aspects of the report is that in COS-7 cells transfected with the receptor DNA containing the mutation, ligand-independent accumulation of cyclic adenosine monophosphate was observed. This was not found in control cells that had been transfected with the wild-type (normal) receptor cDNA.

Schipani E, et al. *Science* 1995;268:98-100.

***Editor's comment**: The etiology of the hypercalcemia in JMC, which is not seen in the Schmidt-type metaphyseal chondrodysplasia, has always been somewhat controversial. Endocrinologists suspected that it was due to hormonal causes, while chondrodysplasia experts, who were mainly geneticists, considered it to be secondary to the severe metaphyseal abnormalities. It is ironic that the endocrinologists used genetic technology to demonstrate that they were correct.*

JMC now joins a growing list of genetic disorders resulting from mutations that activate receptors. These diseases include polyostotic fibrous dysplasia with or without sexual precocity (McCune-Albright syndrome); rare forms of retinitis pigmentosa; congenital nonautoimmune hyperthyroidism; gonadotropin-independent male precocious puberty (testitoxicosis); a hyperparathyroidism-like syndrome in which there is a defect of the calcium sensing receptor; congenital stationary night blindness; and others. A feature article in GGH *discussing these various receptor defects will be forthcoming sometime in the next year.*

William A. Horton, MD

Genetic Heterogeneity in Multiple Epiphyseal Dysplasia

Multiple epiphyseal dysplasia (MED) is an autosomal dominant chondrodysplasia characterized by mild to moderate shortness of the limbs, waddling gait, genu valgum, and early onset osteoarthritis. Two autosomal dominant types have been described: the severe Fairbank type and the milder Ribbing type. Although previously it was suspected that variability occurred within the same disorder, ie, allelic disorders, linkage analysis has shown that there are at least 2 forms: Fairbank-type MED maps to chromosome 19, which may be allelic with pseudoachondroplasia; Ribbing-type MED maps to chromosome 1 near the locus for the alpha 2 chain of type IX collagen (COL9A2). These have been designated EDM1 and EDM2, respectively.

The current report describes 2 intriguing families with clinically similar findings in certain respects and dissimilar findings in others—particularly stature and mode of inheritance.

In family 1, the proband (a 5-year-old female) and 2 siblings (an 8-year-old sister and a 12-year-old brother) presented for genetic evaluation because of painful hips and waddling gait. The heights of these children were not short (35th through 70th percentiles). None were disproportionate since the arm spans approximated the heights. Radiographic findings for all 3 subjects showed typical features of Fairbank-type MED, as the epiphyses were small, irregular, and flat, especially at the knees. The femoral necks were short and broad and the capital femoral epiphyses were small and round. The bones of the hand were normal, but with delayed ossification of the distal ulnar epiphyses and carpal bones. An autosomal dominant inheritance pattern was unequivocal. The authors demonstrated that this family had Fairbank-type MED, with linkage to chromosome 19. All affected individuals had heights within ±2 standard deviations (SD).

The 43-year-old white female proband in family 2 was evaluated because of short stature, which was disproportionate in type, and joint pain. The proband was <2 SD in height, and the arm span was short for the length (length = 151.cm; span = 143.5 cm). Two siblings had joint pain and short stature. The parents had no symptoms of MED and were not short. A recessive inheritance or possibly autosomal dominant inheritance with germline mosaicism was responsible. No abnormalities associated with chromosome 19 or with the cartilage-specific candidate collagen genes (COL) were demonstrable. COL9A2 has recently been reported to be linked in one family with autosomal dominant MED.

In summary, the authors confirm that autosomal dominant Fairbank-type MED maps to chromosome 19. However, they also studied another large MED family in which linkage to the chromosome 19 locus was excluded. They further excluded linkage of MED in this family to the chromosome 1 (EDM2) locus using markers for COL9A2. Thus, at least 1 additional genetic locus remains to be identified for conditions having the clinical and radiographic criteria of MED.

Deere M, et al. *Am J Hum Genet* 1995;56:698-704.

***Editor's comment**: With the recent identification of genes and mutations associated with multiple chondrodysplasias and similar disorders, a trend seems to be emerging. Disorders with similar clinical phenotypes involving genes that encode extracellular matrix proteins exhibit considerable genetic heterogeneity. Mutations tend to occur in different genes and at different sites within the same gene. This is not a new observation. Disorders manifesting spondyloepiphyseal dysplasia and MED phenotypes are good examples.*

In contrast, disorders due to mutations of growth factor receptors, such as fibroblast growth factor receptors, exhibit much less heterogeneity. Mutations tend to cluster in relatively few sites, as in achondroplasia, where almost all patients have the same mutation. Time will tell if these impressions are correct.

William A. Horton, MD

A Cluster of Sulfatase Genes on Xp22.3: Mutations in Chondrodysplasia Punctata (CDPX) and Implications for Warfarin Embryopathy

Chondrodysplasia punctata (CDP) refers to a group of skeletal dysplasias characterized by abnormal calcium deposition in regions of enchondral bone formation. This results in the "stippling" of epiphyses, which tends to disappear within the first few years of life. One type of chondrodysplasia punctata is X-linked recessive (CDPX). It is characterized by aberrant bone mineralization, severe underdevelopment of nasal cartilage, and distal phalangeal hypoplasia. The authors demonstrated that some of these patients have an inherited deficiency of a novel sulfatase (arylsulfatase E, or ARSE). However, not all patients with the clinical syndrome have this defect. Other patients have a recessive form of CDP. CDPX shows remarkable phenotypic similarities to 2 well-characterized disease entities involving vitamin K metabolism: warfarin embryopathy and a congenital metabolic error of vitamin K epoxide reductase deficiency. Warfarin embryopathy is caused by the administration of warfarin, an anticoagulant drug, during a critical period of pregnancy: the sixth through ninth weeks. The vitamin K epoxide reductase deficiency disease, also known as pseudowarfarin embryopathy, is a rare autosomal recessive disorder affecting the recycling of vitamin K. By extensively analyzing DNA from overlapping yeast artificial chromosome clones that spanned the critical Xp22.3 region, Franco et al identified 3 adjacent genes that encoded previously unrecognized sulfatase enzymes. Because of predicted structural similarities to arylsulfatases A, B, and C, the novel sulfatase genes were named ARSD, ARSE, and ARSF. The authors concluded that mutations of the ARSE gene account for many cases of CDPX and that the phenotype results from reduced ARSE enzyme activity. Warfarin probably produces a CDPX-like syndrome because it inhibits ARSE activity. The authors demonstrated a significant decrease of ARSE activity and postulated that ARSE activity is inhibited by warfarin. Patients with CDPX had demonstrably deficient ARSE activity. The ARSE gene is mutated in some cases of CDPX. Intriguingly, the congenital deficiency of vitamin K epoxide reductase, the enzyme recycling vitamin K epoxide to vitamin K, produces an identical picture. The striking similarities among CDPX, warfarin embryopathy, and vitamin K epoxide reductase deficiency phenotypes and the evidence that warfarin inhibits ARSE suggest that these disorders are due to abnormalities in the same metabolic pathway but are of different etiologies.

Franco B, et al. *Cell* 1995;81:15-25.

***Editor's comment**: This paper begins to tie together a number of loose ends for biochemists interested in the arylsulfatase family of enzymes, clinicians interested in sorting out the different forms of CPDX and related conditions, and for geneticists interested in the ancestry of the pseudoautosomal region of the X chromosome, which is where not only the gene for ARSE but also the genes for ARSC and ARSD exist. The patients themselves have underdevelopment of nasal cartilage and distal phalangeal hypoplasia, as well as short stature.*

William A. Horton, MD

***2nd Editor's comment**: The saying that if it looks like an elephant and walks like an elephant, then it* is *an elephant may apply to elephants but does not apply to patients with CPD. Drugs obviously can induce enzymatic deficiencies identical to those induced by genetic mutations or the absence of genes.*

Robert M. Blizzard, MD

Trisomy 18, Molecular Studies, Parental Origin and Cell Division in the Extra Chromosome 18 Material

Trisomy 18, or Edwards syndrome, was first described in 1960. It is the second most common autosomal trisomy. Individuals with trisomy 18 present with characteristic facial features, growth retardation, severe mental retardation, clenched hands with overlapping fingers, and renal and cardiac anomalies. Trisomy 18 has an incidence of 0.18% in all clinically recognized pregnancies and, like other autosomal trisomies, is associated with advanced maternal age. The majority of pregnancies with trisomy 18 abort spontaneously, and only 5% survive to birth. The mean survival after birth is 1 to 3 months, and 95% of those born alive die within the first year of life.

The gene or genes responsible for the trisomy 18 phenotype are not known. While the features of trisomy 18 are most often associated with duplication of the entire chromosome, there are a number of cases in which individuals with a partial duplication of chromosome 18 present with the same or similar features. An effort to identify the regions of chromosome 18 that are critical in producing the phenotype was reported by Boghosian-Sell et al, who analyzed 6 patients with partial duplications of chromosome 18. Fluorescent in situ hybridization with DNA-specific probes to chromosome 18 was used to determine the precise duplication in these patients. The clinical features and the extent of the duplication were compared with 4 previously reported partial trisomy 18 patients. This

permitted identification of the regions of chromosome 18 that may be responsible for the clinical features of trisomy 18. They concluded that the critical region lies between 18q12.1-18q21.2 and 18q22.3.

The parental origin of the additional chromosome as well as the cell division leading to trisomy 18 are important for understanding the etiology of trisomy 18 (Fisher et al).

Trisomy 18 occurs because of nondisjunction during cell division (Antonarakis). Nondisjunction occurs during meiosis when a homologous pair of chromosomes has failed to separate during the first meiotic division or when the double-stranded chromosome has failed to separate into single-stranded chromatids at the second meiotic division. Nondisjunction also can occur during mitotic somatic cell division. The result of nondisjunction is an abnormal number of chromosomes (aneuploidy) for a specific chromosome. Other trisomies associated with nondisjunction include trisomy 21 and trisomy 13.

The analysis of inherited DNA markers, restriction fragment length polymorphisms (RFLPs), and microsatellite repeat polymorphisms has allowed for the tracking of the parental origin and thereby the identification of the mechanism(s) leading to the additional chromosome 18 in individuals with trisomy 18 (Antonarakis; Sherman). In the majority of cases, the additional chromosome is maternal in origin and occurs during the second meiotic division. Recently, Fisher et al documented that in 63 cases of trisomy 18, the maternal chromosome was duplicated in 61. Both paternal cases were attributable to a postzygotic mitotic error. Of 54 maternal cases identifiable for testing, 16 were attributable to an error in the first meiotic division, 35 were due to a second meiotic error, and 3 were the result of a postzygotic mitotic error. Of the cases due to first meiotic error, one third lacked recombination, which apparently made them prone to nondisjunction. All maternal errors were associated with advanced maternal age; however, only the examples of nondisjunction in second meiosis were calculated to be statistically significantly increased because of maternal age.

Antonarakis SE. *N Engl J Med* 1991;324:872-876.
Boghosian-Sell L, et al. *Am J Hum Genet* 1994;55:476-483.
Fisher JM, et al. *Am J Hum Genet* 1995;56:669-675.

***Editor's comment**: New molecular techniques allow the tracking of genes and chromosomes in such a way as to give important clues to the mechanisms causing disease. In the case of trisomy 18, just as in Down syndrome, only part of the chromosome seems to produce the abnormal phenotype seen when present in triplicate. Probably lots of the chromosome 18 is either active or not important because only a few bands on the long arm seem to be required. Since the area of the chromosome producing the phenotype has been narrowed, it seems likely to expect that the specific gene(s) will soon be identified. The DNA markers also allow determination that chromosomal errors can occur at many different times. In the case of trisomy 18, maternal second meiosis (while the egg sits waiting to ovulate) seems to be the most vulnerable time for things to go wrong. However, if the chromosomes have not undergone recombination (crossover), they may malsegregate during meiosis I. At this point in time, it is hard to predict how these errors can be prevented, but it is important to know when they occur.*

Judith G. Hall, MD

Thanatophoric Dysplasia (Types I and II) Caused by Distinct Mutations in Fibroblast Growth Factor Receptor 3

Achondroplasia (ACH) is the most common of the chondrodysplasias. Thanatophoric dysplasia (TD) is the most common of the neonatal lethal skeletal dysplasias. Homozygous ACH and TD are comparably lethal. The clinical and radiographic features of the ACH and TD entities are similar except that heterozygous ACH is less severe. Classic features of both syndromes include micromelic shortening of the limbs; relative macrocephaly with frontal bossing; reduced height of the vertebral bodies; poor cellular proliferation and column formation in the cartilaginous growth plates of the long bones; and shortened ribs, resulting in a reduced thoracic cavity and a bell-shaped abdomen.

Based primarily upon specific radiologic differences, newborns with TD have been classified as having either type I or type II. Those with TD type I have curved, short (telephone receiver-shaped) femora with or without cloverleaf skull deformity. Those with type II TD have relatively longer and straighter femora and the cloverleaf skull deformity is constant.

When mutations of the fibroblast growth factor receptor 3 (*FGFR3*) gene were identified in ACH, the search was on for *FGFR3* mutations in TD. The highest levels of expression of *FGFR3* are in the cartilage growth plates and central nervous system; lower levels of expression are seen in the lung, intestine, and kidney. Because of the striking phenotypic similarities between homozygous ACH and TD and because of recent evidence demonstrating an important role for FGFRs in skeletal development, the investigators extensively analyzed *FGFR3* in individuals with TD to determine if mutations in this gene cause one or more forms of this severe skeletal dysplasia. In the present paper, 22 out of 39 TD type I patients harbored amino acid substitutions in the *extracellular* domain of FGFR3 at codon 248. In contrast, a heterozygous mutation of codon 650 in all 16 cases of TD type II was found. All of these had a lysine in the intracellular tyrosine kinase domain of the receptor replaced by glutamic acid. None of the TD mutations were identified in normal individuals. Moreover, no mutations were detected in parental DNA from 3 sets of parents tested (1 set from a TD type I patient and 2 sets from TD type II patients). This demonstrates the sporadic nature of the mutations.

Tavormina PL, et al. *Nature Genet* 1995:9;321-328.

***Editor's comment:** This paper settles several long-standing debates regarding TD and ACH. FGFR3 is involved in both instances. Different heterozygous mutations are responsible at the FGFR3 locus to produce TD types I and II and ACH. An*

intracellular domain mutation is responsible for TD type II, a mutation of the transmembrane domain is responsible for ACH, and a mutation in the extracellular domain is responsible for TD type I. While the story unravels, it becomes more complex. This report does demonstrate conclusively that the TD phenotypes result from new heterozygous mutations at the FGFR3 locus. It also confirms the long-standing hypothesis that TD and ACH are biologically related and are, in fact, allelic disorders. Finally, the paper substantiates the existence of 2 distinct forms of TD and identifies a biologic basis for the difference. The rapidity with which the story is unfolding offers hope that the end is in sight, but don't hold your breath.

William A. Horton, MD

Pelvic Ultrasonography: Early Differentiation Between Isolated Premature Thelarche and Central Precocious Puberty

The authors previously reported the normal increases in uterine volume/length and ovarian volume that occur during normal growth and sexual development (*Pediatr Radiol* 1994;24: 11-13). In the current article, measurements in girls with premature thelarche (PT) and central precocious puberty (CPP) are reported. The sensitivity (the probability that a test result will be positive when the disease is present) and the specificity (the probability that a test result will be negative when the disease is not present) were compared with ultrasonographic measurements and indices of hormonal control. Fifty-five children with PT between the ages of 0.3 and 7.4 years who were followed for 18 months without progressive sexual maturation and 20 subjects between 2.1 and 7.7 years of age with CPP were studied for uterine length/volume and ovarian volume. All measurements were significantly greater in patients with CPP, compared with patients with PT. No significant differences were found between children with PT and the control group. No overlap was observed in uterine volume between the CPP and PT groups. The sensitivity and specificity values for CPP versus PT were as follows:

Parameter	Sensitivity (%)	Specificity (%)
Uterine volume	100	100
Uterine length	90	100
Ovarian volume	82	95
Peak LH/FSH after GnRH	33	100

FSH, follicle-stimulating hormone; GnRH, gonadotropin hormone-releasing hormone; LH, luteinizing hormone

The authors conclude that measurement of uterine volume is a sensitive method for differentiating girls with PT from those with CPP, in contrast to the use of the vaginal smear and reversal of the serum LH:FSH ratio following LH-releasing hormone stimulation.

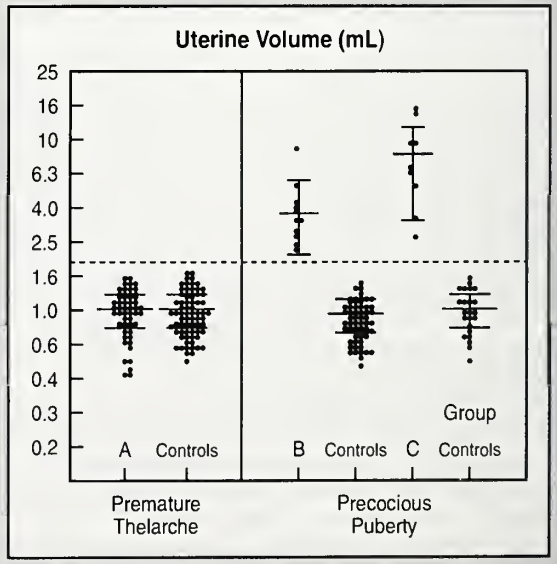
Haber HP, et al. *Eur J Pediatr* 1995;154:182-186.

***Editor's comment**: Distinguishing girls with PT and those with early CPP may be difficult during the initial evaluation. Clinical findings such as the growth pattern, the height, and the degree of sexual maturation assist in differentiating the diagnoses. Haber et al suggest that measurement of uterine volume, which is an index of estrogen activity, distinguishes between PT and CPP. However, pelvic ultrasonography is inconvenient and expensive, although noninvasive. Rectal examinations to determine uterine size and the possibility of ovarian masses were performed for many years before ultrasonography was developed, and worked well. In 1995, physicians should routinely be using the simple tests and resorting to ultrasonography as a backup. Incidentally, uteri are not felt in normal female children from shortly after birth to the onset of secondary sexual characteristics. PT patients usually do not have palpable uteri.*

It was surprising that ovarian volume was not as sensitive or specific a distinguishing characteristic as uterine volume, since gonadal enlargement is presumably the earliest response to gonadotropin stimulation. I suspect that with more experience even the measurement of uterine volume will prove to be less specific and sensitive than reported here, given the broad spectrum of pituitary-ovarian function noted in young girls. Regardless, the use of ultrasonography to supplement the rectal examination when necessary is appropriate.

Allen W. Root, MD

Figure 1



Uterine volume (mean ± SD) in various groups of patients and in related age-matched control subjects. The cutoff value of 1.8 mL is indicated by the *dashed line.*

Metabolic Modulation of the Growth Hormone-Releasing Activity of Hexarelin in Man

Maccario and colleagues studied the mechanism of action of hexarelin by investigating its interaction with glucose and free fatty acids. They specifically questioned whether the growth hormone (GH)-releasing effect of hexarelin could be reduced by factors known to inhibit basal and GH-releasing hormone (GHRH)–stimulated GH secretion. Six normal men participated in the study and underwent 6 treatment sessions separated by washout periods of at least 3 days. All subjects participated in each of the 6 different protocols, which included: (1) hexarelin, 2 µg/kg IV at 0 minutes; (2) GHRH, 2 µg/kg IV at 0 minutes; (3) hexarelin, 2 µg/kg IV at 0 minutes plus glucose (100 g orally at -45 minutes); (4) hexarelin, 2 µg/kg IV at 0 minutes plus lipid-heparin infusion (250 mL of a 10% lipid solution plus 2,500 U heparin from -30 to +120 minutes); (5) GHRH, 2 µg/kg IV at 0 minutes plus glucose; and (6) GHRH, 2 µg/kg IV at 0 minutes plus lipid-heparin infusion. Blood samples were taken every 15 minutes from -60 to +120 minutes. Serum GH, plasma glucose, and plasma free fatty acid levels were measured.

No significant decreases in basal GH were observed during the study. Hexarelin induced a much higher GH peak than did GHRH (62.6 ± 8.0 µg/L vs 19.8 ± 2.4 µg/L). The increase in plasma glucose after the oral load was similar during hexarelin and GHRH testing, but the GH-releasing effect of GHRH was more inhibited by glucose (peak, 5.6 ± 0.9 µg/L vs 38.4 ± 7.9 µg/L) than that of hexarelin. The lipid-heparin infusion increased plasma free fatty acids similarly during both hexarelin and GHRH treatment and basal GH levels were reduced similarly during both studies. The GH released by stimulation with GHRH was reduced to 4.9 ± 1.0 µg/L ($P<0.01$) while that of hexarelin was reduced to 34.2 ± 4.5 µg/L ($P<0.05$). The GH response to hexarelin after glucose was similar to that during lipid-heparin infusion and much higher than the GH response after GHRH alone ($P<0.05$).

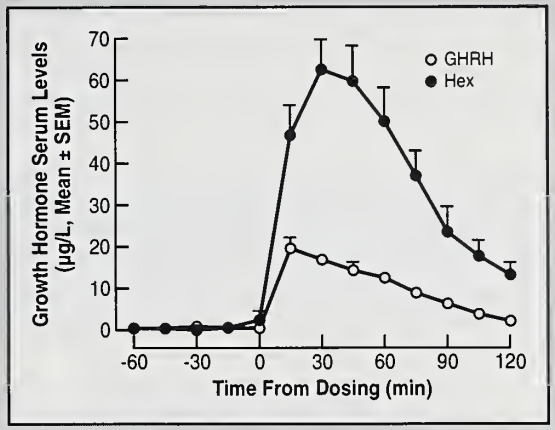
This study demonstrates a greater effect of oral glucose and lipid-heparin infusion on the GH-releasing effect of GHRH than that of hexarelin. The authors state that the results showing that the GH response to hexarelin is blunted but not abolished by glucose indicate that the stimulating effect of GHRP is partially resistant to an increase in endogenous somatostatin. The potential inhibitory effect of free fatty acids on basal and GHRH-induced GH secretion may be explained by a direct action on the pituitary. The GH-stimulating effect of hexarelin is partially resistant to the inhibitory effect of free fatty acids. Thus, unlike GHRH, the GH-releasing effect of hexarelin is partially resistant to the inhibitory effects of both glucose or free fatty acids. This resistance may be due to antagonism of somatostatinergic activity within the hypothalamus or directly at the pituitary. The authors caution that other unknown mechanisms cannot be ruled out.

Maccario M, et al. *Metabolism* 1995;44:134-138.

***Editor's comment**: More and more information is rapidly becoming available regarding the actions of GH-releasing peptides. As the authors point out, GH-releasing peptides release more GH than GHRH, and apparently have some action on specific non-GHRH, nonopiate receptors in both the pituitary and hypothalamus. The present study provides information with regard to the mechanism of action of these hormones and the level at which these hormones may act. Hexarelin is one of the most recently and most frequently studied GH-releasing peptides in humans. It can be given either IV, subcutaneously, intranasally, or orally. We anticipate reports of the use of this hormone to treat patients with GH deficiency due to hypothalamic abnormalities. It is realistic to assume that such data should soon be forthcoming and that GH-releasing peptides may provide a new and potentially more practical method than GHRH for treating some children with growth failure.*

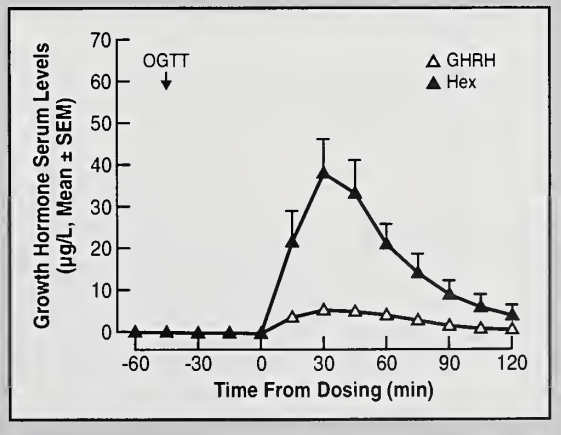
William L. Clarke, MD

Figure 1



Growth hormone responses to hexarelin ([HEX] 2 µg/kg IV) or growth hormone-releasing hormone ([GHRH] 2 µg/kg IV) in 6 healthy men.

Figure 2



Growth hormone responses to hexarelin ([HEX] 2 µg/kg IV) or growth hormone-releasing hormone ([GHRH] 2 µg/kg IV) administered in combination with oral glucose (100 g) in 6 healthy men. OGTT, oral glucose tolerance test.

Recommendations for Standardized Human Pedigree Nomenclature

Significant inconsistencies in the usage of common pedigree symbols lead to inaccurate reporting and poor interpretation of genetic events. Consequently, a Pedigree Standardization Task Force (PSTF) was established by the National Society of Genetic Counselors, and input was solicited from the American Board of Medical Genetics and the American Society of Human Genetics, among others. The article clarifies and standardizes the symbols to be used to describe almost any familial relationship, and also demonstrates specifically how each symbol should be used. Consistent use of such standardized pedigree nomenclature will reduce the chances for incorrect interpretation of patient, family, medical, and genetic information. It also will improve the quality of patient care and facilitate communication among researchers.

Figure 1
Common Pedigree Symbols, Definitions, and Abbreviations

Instructions:
– Key should contain all information relevant to interpretation of pedigree (eg, define shading)
– For clinical (nonpublished) pedigrees, include:
  a) family names/initials, when appropriate
  b) name and title of person recording pedigree
  c) historian (person relaying family history information)
  d) date of intake/update
– Recommended order of information placed below symbol (below to lower right, if necessary):
  a) age/date of birth or age at death
  b) evaluation
  c) pedigree number (eg, I-1, I-2, I-3)

	Male	Female	Sex Unknown	Comments
1. Individual	b. 1925	30 y	4 mo	Assign gender by phenotype
2. Affected individual				Key/legend used to define shading or other fill (eg, hatches, dots, etc)
				With ≥2 conditions, the individual's symbol should be partitioned accordingly, each segment shaded with a different fill and defined in legend
3. Multiple individuals, number known	5	5	5	Number of siblings written inside symbol (affected individuals should not be grouped)
4. Multiple individuals, number unknown	n	n	n	"n" used in place of "?" mark
5a. Deceased individual	d. 35 y	d. 4 mo		Use of cross (†) may be confused with symbol for evaluated positive (+); if known, write "d." with age at death below symbol
5b. Stillbirth (SB)	SB 28 wk	SB 30 wk	SB 34 wk	Birth of dead child with gestational age noted
6. Pregnancy (P)	P LMP: 7/1/94	P 20 wk	P	Gestational age and karyotype (if known) below symbol; light shading can be used for affected and defined in key/legend
7a. Proband	P	P	P	First affected family member coming to medical attention
7b. Consultand				Individual(s) seeking genetic counseling/testing

Because the information is so pertinent, 2 of the numerous figures in the article are reproduced here to encourage interested readers to obtain a complete copy of the article and the inclusive figures for their own use.

Other important figures in the article include a systematic presentation of pedigree line definitions; assisted reproductive technology symbols and definitions; pedigree symbolization of genetic evaluations/testing information; and a hypothetical clinical pedigree, using recommended nomenclature.

***Editor's comment**: Reviewing charts or pursuing the literature about family trees, etc, reveals many inconsistencies in symbols and other designations used in constructing pedigrees. This paper provides needed guidelines for standardization. Pediatric endocrinologists, geneticists, and all pediatricians need to at least understand the symbology used in constructing and reading genetic pedigrees. Obviously, students and residents similarly need this information. It should be incorporated into appropriate teaching programs for all involved medical personnel.*

Bennett RL, et al. *Am J Hum Genet* 1995;56:745-752.

William A. Horton, MD

Figure 2
Pedigree Symbols and Abbreviations for Pregnancies Not Carried to Term

Instructions:
– Symbols are smaller than standard ones and individual's line is shorter. (Even if sex is known, triangles are preferred to a small square/circle; symbol may be mistaken for symbols 1, 2, and 5a/5b of Figure 1, particularly on hand-drawn pedigrees.)
– If gender and gestational age known, write below symbol in that order.

	Male	Female	Sex Unknown	Comments
1. Spontaneous abortion (SAB)	male	female	ECT	If ectopic pregnancy, write ECT below symbol
2. Affected SAB	male	female	16 wk	If gestational age known, write below symbol; key/legend used to define shading
3. Termination of pregnancy (TOP)	male	female		Other abbreviations (eg, TAB, VTOP, Ab) not used for sake of consistency
4. Affected TOP	male	female		Key/legend used to define shading

Chronic Metabolic Acidosis Decreases Albumin Synthesis and Induces Negative Nitrogen Balance in Humans

Ballmer et al measured the effects of experimentally induced metabolic acidosis on nitrogen balance and protein synthesis in 8 male subjects on a constant metabolic diet. Two different degrees of chronic metabolic acidosis were induced using low-dose NH_4Cl (2.1 mmol/kg body weight; n=4) and high-dose NH_4Cl (4.2 mmol/kg body weight; n=4) orally for 7 days. Albumin synthesis rates were determined by a labeled phenylalanine technique after an overnight fast. Urinary nitrogen excretion was measured, as well as plasma concentrations of insulin-like growth factor 1 (IGF-1), free thyroxine (fT_4), and triiodothyronine (T_3).

In the low-dose group, a mean pH of 7.375 and a mean bicarbonate level of 19.1 mEq/L were achieved. The plasma albumin concentration did not decrease significantly. Albumin synthesis in 3 of the 4 subjects was slightly lower than during the control period and definitely decreased in the fourth subject. Nitrogen excretion averaged 977 ± 116 mmol/24 h during the control period and increased, but not significantly, with NH_4Cl administration.

In contrast, plasma albumin concentrations fell significantly in the high-dose group, in whom a mean pH of 7.303 ± 0.053 occurred, in addition to a significantly lower plasma bicarbonate level of 15.1 vs 19.1 mEq/L. Albumin synthesis was significantly lower than during baseline in the high-dose group, and nitrogen excretion increased significantly from 1,012 ± 180 mmol/24 h to 1,377 ± 236 mmol/24 h ($P<0.001$). Plasma levels of IGF-1, fT_4, T_3, and thyrotropin all showed small but statistically significant declines during acidosis, but only when the low- and high-dose groups were combined.

The authors state that these data demonstrate for the first time that metabolic acidosis in humans decreases albumin synthesis and induces a state of sustained negative nitrogen balance. Thus, as stated by the authors, metabolic acidosis could be an important mediator of negative nitrogen balance,

increased protein breakdown, and decreased protein synthesis in acidotic patients. The effect of acidosis on albumin synthesis could be mediated in part by suppression of IGF-1, fT_4, and T_3.

Ballmer PE, et al. *J Clin Invest* 1995;95:39-45.

***Editor's comment**: This is a very interesting and provocative study. More and more pediatric endocrine clinics are treating children with chronic acidosis from chronic renal failure with recombinant human growth hormone. However, the mechanism for the reduction in growth during chronic acidosis remains unclear. This paper contributes to a better understanding of the possible mechanisms involved in growth failure in these children, ie, negative nitrogen balance and decreased albumin synthesis. In addition, it is important to note that, especially in the low-dose group, the reductions in pH and bicarbonate levels were not great, but were comparable to those seen in conditions such as renal tubular acidosis. The number of patients studied in each group was small. Therefore, the trends observed in the low-dose group may be significant with a larger number of subjects.*

William L. Clarke, MD

Endocrinology of the Carbohydrate-Deficient Glycoprotein Syndrome Type 1 From Birth Through Adolescence

Carbohydrate-deficient glycoprotein (CDG) syndrome type 1 is a newly recognized inborn error of glycoprotein metabolism (Jaeken et al. *Acta Pediatr Scand* 1991;375[suppl]:1-71. The biochemical hallmark is a partial carbohydrate deficiency in a wide range of glycoproteins, including binding proteins, enzymes, and coagulation factors. The clinical picture is dominated by the affliction of the central and peripheral nervous system, resulting in psychomotor retardation, seizures, ataxia, and stroke-like episodes. An abnormal pattern of subcutaneous fat occurs, along with feeding difficulties, retinitis pigmentosa, hypoalbuminemia, pericardial effusion, and/or ascites. The diagnosis is confirmed by isoelectric focusing of serum sialotransferrins. CDG syndrome type 1 and type 2 are etiologically distinctly different. This report of 26 CDG type 1–affected children presents pertinent endocrine data.

Serum follicle-stimulating hormone (FSH) levels were normal in newborns and prepubertal children, but elevated in female toddlers and adolescent females and males with CDG syndrome type 1. Serum luteinizing hormone (LH) was similar and was age-dependent. In adolescent girls, serum estradiol remained low while FSH bioactivity was low normal, as was the bioactive/immunoreactive FSH ratio. Exogenous gonadotropins evoked an estradiol response and induced ovarian follicular growth. Male patients virilized at puberty, although testicular volume was subnormal. The thyroid axis was hallmarked by thyroid-binding globulin (TBG) deficiency and, during infancy, increased serum thyrotropin concentrations were observed. A subgroup of female patients presented with hypersomatotropism and/or hyperprolactinemia. The hypothalamic pituitary area appeared intact on magnetic resonance imaging. Circulating insulin-like growth factor 1 (IGF-1) levels were low normal and transcortin levels were decreased.

The etiology is an inherited metabolic error in the posttranslational glycosylation of a variety of glycoproteins. The primary defect for CDG syndrome type 1 may be located at the level of the endoplasmic reticulum. The data are compatible with impaired in vivo function of FSH.

de Zegher F, Jaeken J. *Pediatr Res* 1995;37:395-401.

***Editor's comment**: Abnormalities related to defective glycosylation during posttranslational processing of synthesized proteins expand the list of molecular faults that one must consider when confronted with atypical clinical problems. Glycosylation is essential for normal function of secreted hormones, carrier proteins, and receptors. The defects in vivo of FSH function in children with CDG syndrome type 1 is most striking. Decreased glycosylation of FSH resulted in a prolonged half-life, perhaps leading to downregulation of gonadal FSH receptors and impaired ovarian and testicular responsiveness. Exogenous (glycosylated) FSH reversed the endocrine (ovarian) abnormalities, possibly because the endogenous FSH was suppressed, permitting the receptors to recycle to the plasma membrane and become responsive to stimulation once more.*

The question must be raised whether there are children with less complete defects in glycosylation than those present in CDG syndrome type 1, such as those with an unexplained and persistent modest rise in thyrotropin found during neonatal screening for congenital hyperthyroidism. Possibly there are patients with hypergonadotropic hypogonadism without a specific identifiable primary gonadal abnormality who have a glycosylation defect. The authors of this paper suggest that defective glycosylation and impaired activity of FSH may be present in females with galactosemia and possibly in some women with the hyperandrogenism/polycystic ovary syndrome. A new form of diseases has been encountered and more of this type will probably be described.

Allen W. Root, MD

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc. The information in this publication reflects the views of the editors and/or contributors and not necessarily those of the grantor or the publisher.

Published by:
Gardiner-Caldwell
SynerMed
405 Trimmer Road
PO Box 458
Califon, NJ 07830

Telomeres and Telomerase: Cancer, Immortality, and Mental Retardation

The word telomeres comes from the Greek "telos," which means "end." When applied to chromosomes, it means the end tip of a chromosome. Repetitive DNA sequences (TTAGGG) are located at the end or tip of a chromosome and are called telomeric sequences. Telomeric repeats are highly conserved, with the same sequences found in protozoa, nematodes, lower and higher plants, and vertebrates. Telomeres were first recognized as short repeated sequences at the end of ciliate chromosomes and in lower eukaryotes such as yeast. These repetitive sequences were later recognized and documented in human chromosomes.

Recent evidence has shown that telomeres are involved in a large number of biologic functions. Two among those suggested are very important: (1) protection of the linear chromosome end from degradating, recombining, and ligating to other chromosome ends; and (2) completion of the replication of chromosome DNA sequences at the chromosome ends (Biessman and Mason).

The cloning and characterization of the repetitive sequences that make up human telomeres have greatly benefited from new DNA cloning techniques and have led to interesting observations regarding cancer and aging. For example, the length of a telomere, ie, the number of repetitive sequences, is known to be associated with the number of cell divisions that particular cell has gone through. Telomeres in human germline cells, eg, sperm and egg, are known to be longer than those seen in somatic tissue cells, such as in blood. The telomeres of the human chromosomes shorten with each cell division. Shortened telomeres (in comparison with those of adjacent nontumor mucosa) have been documented in Wilms' tumors and colorectal carcinomas. The telomeric hypothesis (originally called the marginotomy theory) stated that the gradual loss of chromosome ends leads to cell arrest. This theory was based on progressive telomeric shortening with aging and on the observation that if a telomere became too short, cell growth would arrest.

The enzyme that synthesizes the telomeric sequences is a ribonucleoprotein enzyme called telomerase. Telomerase has been shown to be abnormally increased in some cancer cells. The gene for telomerase in humans has not been mapped. Telomerase expression is directly related to telomeric conservation. Excessive expression of telomerase has the potential to stop or delay the normal shortening of the telomeres and, consequently, delay cell cycle arrest. Aberrant telomerase expression has been suggested as a mechanism for producing the "immortality" of cancer cells.

The shortening of telomeres of human chromosomes with each cell division has been thought to serve as some sort of mitotic clock that can be used as a direct marker for the number of times a cell has divided. The exact role shortened telomeres play in aging is still unclear; however, telomeric loss with a successful series of cell divisions has been referred to as a "genetic time bomb" (Harley), since it will eventually lead to cell death.

An abnormality of telomeres also has been associated with mental retardation. In a recent report, Flint et al studied the subtelomeric regions of 99 mentally retarded individuals. They hypothesized that since the telomeric end of the chromosome is an area of active recombination, it would be expected to be at risk for small deletions. To detect chromosomal abnormalities within the subtelomeric region, they used hypervariable DNA polymorphism probes. They compared the DNA of both parents with the mentally retarded offspring. They found that 3 of 99 patients had abnormalities. One arose from an interstitial or terminal deletion and 2 from the de novo derivative translocation of 2 chromosomes. They suggest that at least 6% of all unexplained mental retardation may be the result of these small telomeric abnormalities.

***Editor's comment**: Telomeres have been studied for many years but only lately have we become aware that they are involved in much more than just making up the ends of a chromosome. Telomerase expression may provide the means for diagnosing cancer or identifying the presence of malignant cells. Downregulating telomerase as a molecular therapeutic intervention may be applicable to a wide range of cancers. The findings of Flint et al address a different aspect of telomeres. The suggestion that as many as 6% of cases of idiopathic mental retardation can be explained by a telomeric loss provides a new and important diagnostic tool in mental retardation for families with previously unexplained mental retardation who are concerned about the risk of recurrence. Looking for telomere abnormalities may allow a definitive diagnosis with a low recurrence risk for the parent but with as much as a 50% risk to the offspring of the affected individual. It will now be necessary to counsel families that a search for telomeric loss may be appropriate in nonspecific mental retardation.*

Judith G. Hall, MD

Biessmann H, Mason JM. *Adv Genet* 1994;30:185-249.
Flint J, et al. *Nature* 1995;9:132-138.
Harley CB. *Mutation Res* 1991;256:271-282.

***2nd Editor's comment:** Daniel Haber discussed the topic of telomeres, cancer, and immortality in a brief commentary in the* New England Journal of Medicine *(April 6, 1995). He states that, among other things, the progressive shortening of telomeres correlates with the absence of expression of telomerase and that continuing expression of telomerase correlates with the presence of cancer cells. In humans, germ cells express telomerase and maintain their ability to divide throughout life. In other cells, an estimated 15 to 40 nucleotides are lost each year. Kim et al (*Science *1994;266:2011) demonstrated that 90 of 101 specimens from primary tumors representing 12 different types of cancer contained telomerase activity, in contrast to none of 50 normal tissues. The extreme sensitivity of the polymerase chain reaction–based enzymatic assay allows the detection of 1 cancer cell expressing telomerase among 4,000 normal cells. Haber points out that an effective inhibitor of telomerase might induce prompt senescence in rapidly dividing tumors. Whether clinical applications will be forthcoming in the near future is unknown at this time. You, the reader are urged to learn more about telomeres and telomerase. Haber's commentary is a good place to start. The references listed above are excellent as follow-ups. Dr. Hall has done her usual proficient job in calling these phenomena to our attention.*

Robert M. Blizzard, MD

September 27-30, 1995 Molecular and Developmental Biol of Cartilage, Bethesda, MD. Info: Conf Dept, NY Acad Sci. Tel: 212-838-0230, ext. 324; Fax: 212-838-5640.

October 18-20, 1995 Intl Symp on Growth, Santiago de Compostela, Spain. Info: Prof FF Casanueva, C Dieguez, or M Pompo. Fax: 34-81-572-121.

October 24-28, 1995 45th Ann Mtg of the Amer Soc of Human Genet, Minneapolis, MN. Info: M Ryan. Tel: 301-571-1825; Fax: 301-530-7079.

November 8-11, 1995 APS Conf: New Discoveries Within the Pancreatic Polypeptide Family: Molecules to Medicine, Location TBA. Info: Amer Physiol Soc, Membership Services. Tel: 301-530-7171; Fax: 301-571-8305.

November 25-28, 1995 17th Mtg of the Intl Study Group for Steroid Hormones, Berlin, Germany. Info: Dr V Toscano. Tel: 39-6-494-0568; Fax: 39-6-490-530.

December 9-13, 1995 35th Ann Mtg of the Amer Soc for Cell Biol, Washington, DC. Info: ASCB. Tel: 301-530-7153.

January 5-11, 1996 Integrins and Signaling Events in Cell Biol and Disease, Keystone, CO. Info: Keystone Symposia. Tel: 303-262-1230; Fax: 303-262-1525.

January 5-11, 1996 Small GTP-Binding Proteins and Growth Factor Signaling Pathways, Tamarron, CO. Info: Keystone Symposia. Tel: 303-262-1230; Fax: 303-262-1525.

January 7-10, 1996 6th Wkshp on Cells and Cytokines in Bone and Cartilage, Davos, Switzerland. Info: H Triet. Tel: 41-31-632-8766 or 41-31-632-2518; Fax: 41-31-382-3038; Email: pphysecrpphy.unibe.ch.

January 11-13, 1996 3rd Wkshp on Bisphosphonates, Davos, Switzerland. Info: H Triet. Tel: 41-31-632-8766 or 41-31-632-2518; Fax: 41-31-382-3038; Email: pphysecrpphy.unibe.ch.

March 11-14, 1996 Amer Coll of Med Genet and the March of Dimes, San Antonio, TX. Info: Amer Coll of Med Genet, M Ryan. Tel: 301-571-1825.

April 13-16, 1996 Amer Academy of Ped Mtg, Chicago, IL. Info: M Francis. Tel: 800-433-9016; Fax: 708-228-5059.

May 6-9, 1996 Amer Ped Soc/Soc for Ped Res Mtg, Washington, DC. Tel: 708-427-0206; Fax: 708-427-1305.

May 16-19, 1996 Review Course for Genetics, Houston, TX. Info: Office of Cont Ed, 4 Beaudet. Tel: 713-798-6020; Fax: 713-798-7955.

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc.

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GROWTH
Genetics & Hormones
Vol. 11 No. 4 December 1995

Letter From the Editor

To Our Readers:

This issue of *GROWTH, Genetics, & Hormones (GGH)* is comprised, to a significant extent, of a comprehensive index for *GGH* Volume 1, Number 1 (1985) through Volume 10, Number 4 (1994). Many readers have requested this because *GGH* has evolved as an educational tool for teaching residents and fellows, as well as a reference journal for review articles presenting new information and concepts abstracted from the most recent journal publications. Hopefully, each of you will find this index to be valuable.

We look forward to serving you in 1996. We, the Editorial Board, appreciate, as we are sure you do, the unrestricted educational grant from Genentech, Inc. that makes this publication possible.

For the Editorial Board,

Robert M. Blizzard, MD
Chairman, *GGH* Editorial Board

Letter to the Editor

In the most recent issue of *GROWTH, Genetics, & Hormones* (*GGH* 1995;11[3]:12-13), the new recommendations for standardized human pedigree nomenclature were presented as a summary of an article by Bennett RL et al (*Am J Hum Genet* 1995; 56:745-752). The summary included a figure from the article with symbols and definitions.

For the most part, the recommendations in the Bennett et al article are excellent and will go a long way toward standardizing pedigree nomenclature. However, there is one exception: the definition of "proband" as "the first affected family member coming to medical attention" (item 7a in the figure published in *GGH* on page 12). This definition is more properly that of "index case." The term "proband" has a specific meaning, which is different from the Bennett et al definition. One difference, for example, is that there can be only one index case per pedigree, whereas there can be, and often is, more than one proband per pedigree. It has been well documented that proband misidentification can introduce serious bias into any analysis of family data.

I made these points in a letter to the editor of the *American Journal of Human Genetics*. Bennett et al replied and agreed that the proband definition should be modified. If possible, it would be helpful for a clarification to be published in *GGH*. *GGH* is widely read and respected, and it would help in spreading the word about the change in proband definition from the Bennett et al original definition.

Sincerely yours,

Mary L. Marazita, PhD, FACMG
Director, Cleft Palate-Craniofacial Center
Associate Professor, Oral and Maxillofacial Surgery
Associate Professor, Human Genetics
University of Pittsburgh

Editor's comment: Thank you, Dr. Marazita, for your letter and the constructive criticism of the new recommendations for standardized human pedigree nomenclature. My review of the original article by Bennett et al and their reply regarding your concern concurs that confusion can exist by using the term "proband" instead of "index case." The readers are urged to modify Figure 1 (*GGH* 1995;11[3]:12) as you suggest and to modify their use of the terms "index case" and "proband" as you suggest.

Sincerely,

Robert M. Blizzard, MD
Chairman, *GGH* Editorial Board

In This Issue

Predictive Factors in the Determination of Final Height in Boys With Constitutional Delay of Growth and Puberty

Albanese and Stanhope hypothesized that boys with constitutional delay of growth and puberty (CDGP) form a heterogeneous diagnostic category composed of children with varying degrees of impairment of final height. Consequently, they analyzed the patterns of growth in height and the changes in body proportions in 78 prepubertal or early pubertal boys with CDGP. The characteristics of those in this group were that the chronologic age was ≥13 years and bone age delay was >1.5 years. These boys were treated for 4 months with either 50 mg of sustained-action testosterone every 2 weeks or 1.25 mg daily of oxandrolone, or received neither drug. The mean height standard deviation score (SDS) was -2.7 ± 0.7 (140.6 ± 8.6 cm) at the initial evaluation and -2.0 ± 0.9 (160.5 ± 6.7 cm) at final height. The latter was significantly below either the mean predicted adult height or the corrected midparental height (MPH), although much overlap occurred. The final height of 45 (58%) of the 78 patients did not achieve the target height range. Of the 33 (42%) of patients whose final heights fell within the target height range, the heights of only 3 (0.7%) exceeded the corrected MPH.

At final height, several (26%) of the boys had eunuchoid habitus, with short spines relative to lower limb lengths at diagnosis and at final height. Using multiple regression analyses, the authors determined that standing height, growth velocity, and the difference between the sitting height and the subischial leg length present at the initial evaluation could be used as predictors of impaired final height. Neither the chronologic age, the delay in bone age at the initial examination, nor treatment for 4 months with androgens influenced this analysis.

The authors conclude that decreased spinal growth is present in many boys with CDGP, and that the presence of a short spine relative to leg length suggests that final adult stature will be impaired.

Albanese A, Stanhope R. *J Pediatr* 1995;126:545-550.

***Editor's comment**: As the authors point out, the 78 boys studied represent only a fraction of those with CDGP, and possibly only those with the most severe impairment of growth were followed in the authors' clinic. Therefore, their conclusions may be applicable only to a subset of patients with CDGP. Nevertheless, the observation of impaired prepubertal spinal growth leading to impaired final height prompts the question whether some patients with CDGP have a subtle spinal chondrodystrophy. The report indicates the need to routinely measure sitting heights or upper to lower ratios in such patients.*

Allen W. Root, MD

***2nd Editor's comment**: The authors were unable to explain the failure to achieve target height in 58% of their patients. Speculation is appropriate that this subgroup may have a variant of CDGP, one with growth hormone insufficiency that is not revealed by pharmacologic tests of growth hormone secretion, or they possibly may have an unclassified skeletal dysplasia. The authors also conclude that treatment with androgens, at the doses used, does not improve final height but only accelerates the growth spurt. They do suggest that the use of low doses of oxandrolone may prevent reduced spinal growth and, consequently, improve final height. In this editor's opinion, the problem with some of these speculations is that only 4 months of androgen therapy were used, and this short period of therapy may not affect either predicted height or ultimate height. Earlier androgen therapy over a prolonged period but at a dose absolutely not higher than that recommended by the authors may be beneficial in increasing ultimate height. Studies need to be done concerning this.*

Robert M. Blizzard, MD

Zinc Deficiency in a Breast Fed Premature Infant

Zinc is an essential element for a variety of biochemical functions of the human body, including normal function of skin, the gastrointestinal system, and the immune and central nervous system (CNS) systems. Individuals with severe zinc deficiency present with erosive skin changes, particularly of the face and anogenital area, and with alopecia of scalp hair. Failure to thrive, irritability, and immunodepression are also common.

Several causes of zinc deficiency syndrome are known. These include: (1) deficient exogenous zinc supply, either from breast milk when the mother is deficient in zinc or in her diet; (2) increased intestinal or urinary zinc loss; (3) inadequate absorption in preterm infants; and (4) poor storage. Since breast milk usually is a good source of zinc, severe zinc deficiency in full-term infants is very rare. However, a number of investigators have reported zinc deficiency in breast-fed, preterm infants (Aggett et al; Bilinski et al; Buehning and Goltz).

A recent paper by Heinen et al reports a typical case of a preterm infant who was exclusively breast-fed and suffered from severe zinc deficiency syndrome. The neonatal period was complicated by bronchopulmonary dysplasia, cerebral hemorrhage with subsequent hydrocephaly, and ventriculitis. A zinc-containing formula was given only for the first 4 days of life. After that he was breast-fed and received parenteral nutrition without zinc supplements. At 20 weeks, erosive skin changes, developmental retardation, and muscular hypotension were noted. Blood zinc levels measured in both mother and infant were significantly low. Oral zinc therapy was instituted. Marked improvement in the skin lesions occurred in 2 days. Heinen et al concluded that a diet based exclusively on breast milk may in some cases, depending on the mother's nutritional status, lack sufficient zinc and lead to severe zinc deficiency in the infant.

Aggett P, et al. *Arch Dis Child* 1980;55:547-550.
Bilinski DL, et al. *Arch Dermatol* 1987;123:1221-1224.
Buehning LJ, Goltz RW. *J Am Acad Dermatol* 1993;28:499-501.
Heinen F, et al. *Eur J Pediatr* 1995;154:71-75.

***Editor's comment**: Zinc deficiency is rare without a predisposing disease such as acrodermatitis enteropathica. However, it must be considered in the premature infant who may have less than normal zinc absorption. This particular case was complicated by prematurity and maternal zinc deficiency. He responded really well to therapy. Interestingly, the authors point out that if zinc oxide paste is used for diaper rash, the zinc may be absorbed transcutaneously from the anogenital area.*

Judith G. Hall, MD

***2nd Editor's comment:** Zinc deficiency in premature, breast-fed infants has been previously described in the literature. It is not apparent why the authors state that this case is an example of a "distinct form of zinc deficiency syndrome." The infant described received markedly inadequate zinc intake from day 4 through 21 of life by being treated parenterally without zinc supplementation. The zinc content of the breast milk also was poor: <50% of the usual zinc content of human milk. No information was presented regarding the amount of breast milk that the baby was receiving. Since the infant was failing to thrive, energy requirements of the infant may not have been met secondary to inadequate breast milk feedings.*

No information was given to explain why the mother was zinc deficient. Did she have a genetic disposition to zinc deficiency or possibly a medical condition that interfered with zinc absorption or utilization? Moreover, there is no discussion about the mother's nutritional status during pregnancy. Speculation that the marginal zinc status of the mother may have contributed to the baby's congenital abnormalities is appropriate. In animal models, zinc deficiency during gestation has resulted in teratogenic fetal abnormalities, including neural tube defects. Epidemiologic data also link maternal zinc deficiency and CNS malformations in the fetus. Women with acrodermatitis enteropathica have a high incidence of spontaneous abortions and fetal alterations, including skeletal abnormalities and anencephaly.

Zinc needs of growing infants may be best met by breast milk even when there is intestinal malabsorption. However, low-birth-weight infants may be at risk for zinc deficiency and for these infants, the quality of the breast milk must be considered. Zinc content of human milk normally falls as lactation progresses. Thus, banked human milk may contain less zinc than the mother's own milk and should be analyzed and supplemented with breast milk fortifier before use in feeding premature infants. Providing parenteral nutrition to a premature infant without zinc supplementation is inappropriate medical care. Current recommendations call for 400 mg/kg/d of zinc in total parenteral nutrition solutions. Poor nutrition during gestation will have a great impact on the health of the baby, and knowledge of the mother's nutritional health will assist in treating the infant appropriately.

Fima Lifshitz, MD

Adrenal Insufficiency and Bronchopulmonary Dysplasia in Low Birth Weight Infants

The causes of bronchopulmonary dysplasia are not clear, but it has been associated with injuries due to mechanical ventilation. However, chronic lung disease and bronchopulmonary dysplasia can develop in small premature infants who show little initial respiratory distress and who have never needed respiratory assistance.

Glucocorticoids produced by the adrenal glands play an important role in the resolution of inflammation and the response to stress. The trigger for the release of glucocorticoids from the adrenal glands is the hypothalamic-pituitary-adrenal axis. If this axis is functioning normally, inflammatory reactions are easily resolved and the damage repaired. However, if the axis is not functioning properly, impaired inflammatory reactions can be excessive and damage repair progresses very slowly.

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Some studies done in animal models have shown that in the first few hours of life the response to adrenocorticotropic hormone (ACTH) may be absent (Guillet et al; and Walker et al). Studies in humans show that in the first hours of life some low-birth-weight neonates may also lack the appropriate response mediated by the hypothalamic-pituitary-adrenal axis. This has been called the neonatal stress "nonresponsive" or "hyporesponsive" period. During this period the adrenal gland shows a diminished or absent response to ACTH. This nonresponsiveness is thought to resolve within the first week of life.

A recent study by Watterberg and Scott suggested that some premature infants may not recover from the neonatal stress nonresponsive period as fast as expected and are thus predisposed to greater damage from inflammatory reactions. In order to confirm this, they tested the cortisol response to ACTH by measuring blood cortisol secretion levels in a population of very-low-birth-weight infants.

At the end of the first week of life, the infants who had higher blood cortisol levels recovered without bronchopulmonary dysplasia while the infants with lower cortisol levels eventually developed bronchopulmonary dysplasia and

remained dependent on oxygen supplementation. Watterberg and Scott concluded that the infants in the latter group may not be able to secrete an adequate amount of cortisol in response to stress and thus inflammation goes unchecked and renders them susceptible to long-term lung injuries.

Guillet C, et al. *Endocrinology* 1980;106:991-994.
Walker CD, et al. *Endocrinology* 1986;118:1445-1451.
Watterberg KL, Scott SM. *Pediatrics* 1995;95:120-125.

***Editor's comment**: These observations could have important ramifications for preventive therapy in premature infants. Bronchopulmonary dysplasia is a very crippling disorder and its prevention would certainly be welcome. The observations need to be confirmed. An appropriate method of screening and trial of therapy could then lead to prevention of this dreaded complication of prematurity.*

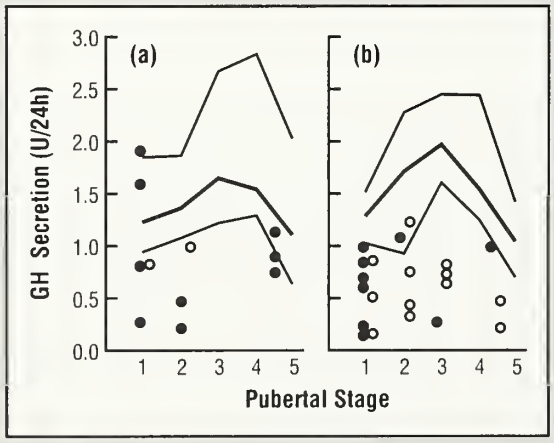
Judith G. Hall, MD

Reduced Growth Hormone Secretion With Maintained Periodicity Following Cranial Irradiation in Children With Acute Lymphoblastic Leukaemia

Lannering et al obtained growth hormone (GH) determinations every 20 minutes for 24 hours in a group of 34 children with acute lymphoblastic leukemia (ALL) who had received cranial irradiation with 18 to 24 Gy. These children (12 boys and 22 girls) had been diagnosed 4 to 10 years previously; their mean age at diagnosis was 3.9 years. Fourteen (5 boys and 9 girls) were prepubertal at the time of the study (using Tanner staging). Height was expressed as standard deviation scores (SDS) in comparison with Swedish reference values for healthy children. A control group of 208 children was utilized. The GH profiles were analyzed using the Pulsar pulse detection program and Fourier time-series analysis.

The estimated GH secretion rate in all irradiated ALL children was below the median of that of controls for pubertal stage and sex. The difference between patients and controls was more pronounced in late puberty than before puberty. GH secretion as expressed by the area under the curve was also reduced in irradiated children. However, the number of GH peaks over 24 hours was within the normal range for both boys and girls. Before puberty a broad range of cycles per 24 hours was seen; these synchronized during puberty to approximately 1 every 3 to 4 hours. Lower peak amplitudes were observed in the irradiated children. There was no correlation between time from diagnosis and GH secretion or the maximal GH level during the 24-hour period. There were no obvious influences of the time of diagnosis on GH secretion. Children who were still prepubertal at the time of the study had lost an average of 0.2 SDS. Children who had entered puberty lost an average of 1.0 SDS.

Figure 1



Individual values of growth hormone (GH) secretion rate are shown for (a) boys and (b) girls with acute lymphoblastic leukemia irradiated with ● (18 Gy) or ○ (24 Gy). GH secretion rate of healthy, normally-growing children at pubertal stages 1 through 5 is also given (75th, 50th, and 25th percentiles).

The authors state that their results indicate not only that cranial irradiation in the range of 20 to 24 Gy alters GH secretion (as determined by Moell et al, 1988), but also that irradiation with 18 Gy both before and during puberty reduces GH secretion. Specifically, there was lower pulse amplitude in the irradiated patients, suggesting a physiologic GH insufficiency. Height of the children at a mean follow-up age of 7 years fell within the normal range for the Swedish population. Final heights were not reached in a majority of patients. The authors further state that the impairment observed in growth is small before puberty. The recommendation is made that ALL patients should be studied repeatedly as adults to evaluate the effects of decreased GH secretion on organs other than the growth plate.

Lannering B, et al. *Clin Endocrinol* 1995;42:153-159.

***Editor's comment:** More and more information regarding the effects of cranial irradiation on pituitary function is becoming known. Although most pediatric endocrinologists recognize that irradiation with 24 Gy could be expected to be associated with pituitary dysfunction, it is not generally felt that lower dosages will be detrimental. However, few investigators have performed the careful type of analysis that Lannering and coworkers presented. Their data suggest that there are indeed significant reductions in GH secretion with smaller doses of radiation that may not be clinically observable (no obvious reduction in stature) until puberty, and that there is little difference between the effects of 18 and 24 Gy. It will be interesting to review final heights in the patients reported in this study. One may then be able to better counsel families whose children have received even modest doses of cranial irradiation.*

William L. Clarke, MD

GROWTH Genetics & Hormones

Index

Longitudinal Data on Growth and Final Height in Diabetic Children

Growth and development were analyzed in 2 cohorts of diabetic children. In one cohort (n=46; 22 girls and 24 boys) children with a mean age at diagnosis of diabetes of 7.5 years were followed until they attained final height; in the other cohort (n=27; 11 girls and 16 boys) diabetic children were followed from less than 7 years of age to age 10 years for evaluation of early pubertal growth. All children were treated with conventional insulin therapy (2 injections a day of both long-acting and short-acting insulin). Metabolic control was assessed by glycosylated hemoglobin A_{1c} (HbA_{1c}). Height (Ht) was evaluated every 6 months and transformed into standard deviation scores (SDS) using the 1966 standards for height developed by Tanner, applying a correction for the secular trend. Onset of puberty, final height, total pubertal height growth, body mass index and skeletal age were also recorded. The whole group of patients showed a mean final Ht SDS lower than Ht SDS at onset of disease (0.27 ± 0.97 vs 0.41 ± 0.99 in girls and 0.48 ± 0.89 vs 0.56 ± 0.68 in boys). Final Ht was significantly lower than target Ht in girls (163.7 ± 5.9 cm vs 167.1 ± 5.0 cm, $P<0.05$) but boys did not have significant differences (177.1 ± 6.1 vs 178.1 ± 6.0 cm).

Prepubertal growth was not affected by diabetes mellitus in either sex, but there was pubertal delay in boys. Ht SDS evolution showed a significant drop over the last 2 years of prepubertal growth in both, associated with the delay in the onset of puberty. Total pubertal height gain was negatively correlated with the chronologic age at onset of puberty in both boys and girls.

Girls gained weight excessively during pubertal growth. Their body mass index SDS increased from 0.26 ± 0.98 at Tanner stage 2 for breast development to 0.69 ± 0.97 at final height. Bone age did not deviate from chronologic age at 10 years and at the time of pubertal stage 2.

The authors concluded the following: (1) Diabetic children have normal height at the onset of their diabetes. (2) Final height in girls was slightly reduced from target height. (3) Diabetic girls had a tendency to become obese during puberty. (4) Boys with diabetes showed a marked delay in the onset of puberty but attained an appropriate final height for their target. No correlation was found between the degree of metabolic control (HbA_{1c}) and the total pubertal height gain.

Du Caju MVL, et al. *Pediatr Res* 1995;38:607-611.

***Editor's comment**: This is a unique paper describing growth and development in a small group of children with diabetes mellitus on a longitudinal basis, from onset of diabetes until completion of growth. Other papers assessing growth in diabetic children are cross-sectional studies, thus yielding data that cannot be as reliable as that described here.*

However, it is hard to understand the precise significance of the authors' findings, since there were no measurements of growth para-factors in this study that could help ascertain possible pathophysiologic mechanisms to explain some of the differences mentioned above. Why is it that girls had a loss of final height whereas boys did not? Also, why did females become obese while males did not?

The authors did not find alterations of growth at the time of disease onset nor did they find a correlation with the degree of metabolic control. Other studies have shown improvement of growth with tight control of diabetes.

Fima Lifshitz, MD

Zinc Supplementation and Growth of Infants Born Small for Gestational Age

Sixty-eight full-term small-for-gestational-age (SGA) infants were randomized into 2 groups: 1 of zinc (Zn) supplemented (S) and 1 of placebo (P) infants, in a double-blind manner. Group S received Zn 3 mg/d PO between feedings as a solution of Zn acetate containing 1 mg Zn/mL; group P received an equivalent volume of placebo solution. Both solutions were added to aspartame to make their taste similar. Data analysis was done with 35 infants in group S and 33 infants in group P. Before starting the Zn supplementation or placebo, and at 30, 60, 120, and 180 days of life, all infants had a blood sample drawn for measurement of plasma Zn; samples of occipital hair for measurement of hair Zn and accurate measurements of length, weight, and head circumference were obtained. All infants were initially breast-fed. Supplementation with cow's milk-based formulas was done at different ages according to perceived needs and not knowing the group of assignment. Weight (Wt)-for-age standard deviation scores (SDS) showed differences in catch-up growth between the 2 groups of patients. Patients in group S showed better catch-up growth than patients in group P. The best improvement in Wt-for-age SDS in relation with Zn supplementation was seen among girls. An additive effect for increased Wt-for-age catch-up growth was seen in infants exclusively breast-fed for 4 months, Zn supplemented, and of the female gender. Length (Lt)-for-age SDS showed similar improvements in both groups. Plasma and hair Zn

decreased in both groups over 6 months, but the decline was less pronounced in group S.

The authors conclude that Zn supplementation during the first 6 months of life of SGA infants in the population studied had beneficial effects for growth in both genders, but was more pronounced in girls. They attributed this effect to a deficiency in Zn nutritional status in SGA.

Castillo-Durán C, et al. *J Pediatr* 1995;127:206-211.

***Editor's comment**: Micronutrients, particularly Zn, have been receiving increasing attention in the last few decades in regards to their role in human growth and development. Zn accretion occurs mainly during the third trimester and has been calculated to be 0.85 mg/d. However, full-term infants born SGA may be at a particular risk for Zn deficiency. The American Academy of Pediatrics Committee on Nutrition has recommended that formulas for full-term infants supply at least 0.5 mg Zn per 100 kcal.[1] Recommendations of up to 1 mg/kg/d have been given for preterm infants in the stable/postdischarge period. The recommendation also includes to adding only 0.5 mg/kg/d when the infant is fed human milk.[2] The authors supplemented these infants with 3 mg/d Zn regardless of whether they were being breast-fed or formula fed; they showed an improved catch-up growth when given Zn supplementation. This paper suggests that the recommendations for Zn supplementation in this group of infants should be reconsidered and would be higher than the current recommendations. This is applicable even to breast-fed patients.*

The difference between girls and boys remains unexplained, and as the authors point out, deserves further exploration.

***2nd Editor's comment**: These infants were SGA infants for the most part, and not IUGR as defined and described by Warshaw in* GGH *(1992;8[1]:5-8), which the readers are encouraged to review. The concern this editor has is the wide SDS seen in both groups at each time point evaluated. A tighter statistical difference may be needed to be certain that the findings were not spurious. Regardless, the data gained may assist in evaluating the role of Zn in relation to growth parameters in SGA infants.*

Robert M. Blizzard, MD

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1. Forbes GB, Woodruff CW, eds. *Pediatric Nutrition Handbook.* American Academy of Pediatrics Committee on Nutrition. Elk Grove Village, IL: American Academy of Pediatrics; 1985.
2. Reifen RM, Zlotkin S. Microminerals. In: Tsang R, et al, eds. *Nutritional Needs of the Preterm Infant.* Pawling, NY: Williams & Wilkins; 1993:195-207.

Effects of Differences in Dietary Fat on Growth, Energy and Nutrient Intake From Infancy to 8 Years of Age

Boulton and Magarey, as part of the ongoing Adelaide (Australia) Nutrition Study, evaluated retrospectively growth and energy intake in a cohort of 140 randomly selected children. Subjects were seen at 3, 6, and 12 months of age and at 2, 4, and 8 years of age. Before each of the visits, the parents kept a record of the child's diet. Up to 2 years of age, a 7-day weighed food record was kept. At 4 years, a 3-day record was kept; and at 6 and 8 years, a 4-day weighed food record was kept. The diet composition was analyzed using a computer program. Energy and nutrient intakes were expressed as mean intake per day. Fatness was evaluated by the sum of 4 skin-fold thickness measurements (left mid-biceps, triceps, subscapular, and suprailiac). At each age, the sample was divided into 3 groups according to the percentage of food energy derived from fat: <30%, 30% to 34.9%, and >35%. These cutoffs were chosen since 30% corresponds to the fat intake target for Australian adults, and 35% is the recommended maximum level of fat intake for young children in some countries.

The authors state that there were no significant differences in energy or nutrient intake or attained height and weight through infancy to 8 years of age according to the proportion of fat in the diet, and those in the low-fat group did not have lower essential mineral intake. They speculate that the boys in the low-fat group at 2 years of age may have had a slower growth velocity, thus accounting for their slightly lower height at age 15. They conclude that a shift to a low-fat intake in early childhood is unlikely to have any deleterious effects on growth.

Boulton TJC, Magarey AM. *Acta Paediatr* 1995;84:146-150.

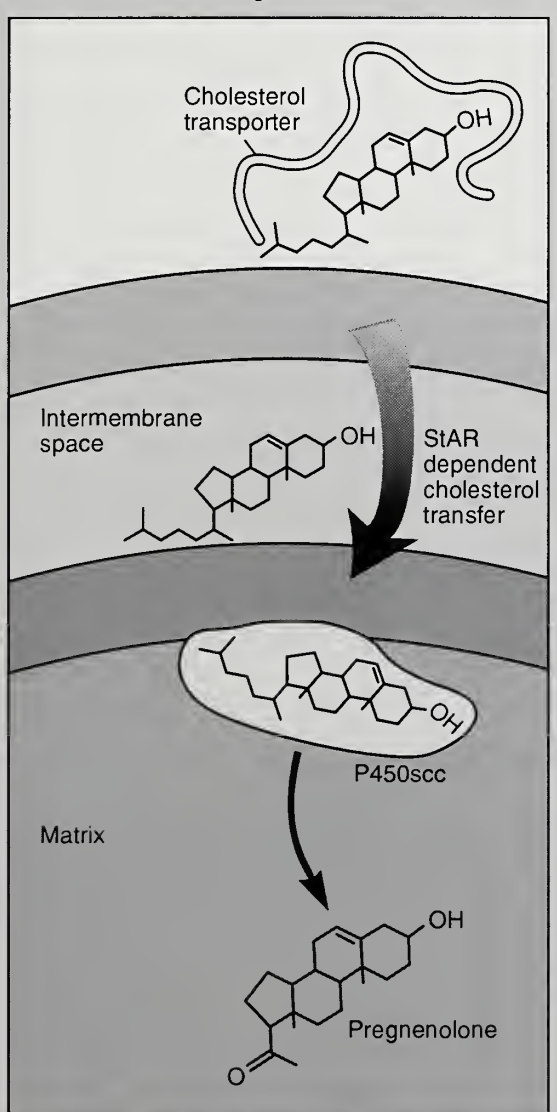
***Editor's comment:** This is a very interesting and important retrospective study. The current dietary recommendation for adults in the United States is to derive <30% of our daily caloric intake from fat. Whether this level of fat intake will have a significant effect on growth and the timing of puberty has been the subject of some controversy. The fact that in the present report boys with lower fat intakes at age 2 were somewhat shorter at age 15 than those with higher fat intakes suggests that there may be some validity to these concerns. However, what is not clear in this study is whether these 140 children remained in the same fat intake group throughout childhood. Children were not randomly assigned to a specific level of fat intake but rather their natural eating habits were evaluated using 4- to 7-day food records. Thus, there is a strong possibility that some children switched from group to group throughout childhood. There is also some concern with regard to the validity of dietary food records, although recent studies suggest that this is a relatively accurate means for measuring nutrient intake. Despite these potential shortcomings, the information in this study is of significant interest and importance to physicians who prescribe dietary regimens for children.*

William L. Clarke, MD

Role of Steroidogenic Acute Regulatory Protein in Adrenal and Gonadal Steroidogenesis

Lin et al identified the molecular defect that causes lipoid adrenal hyperplasia, an autosomal recessive disorder that is associated with feminization of male external genitalia, severe salt loss in both sexes, and excessive amounts of cholesterol and impaired steroidogenesis in the adrenal and gonads. The abnormality was suspected to be in the cholesterol side chain cleavage enzyme (P450scc), but analysis of the P450scc gene (*CYP11A*) has been normal in affected subjects. Lin et al provide evidence that the primary defect is in the steroidogenic acute regulatory (StAR) protein. This mitochondrial protein escorts cholesterol from the interior surface of the outer mitochondrial membrane to the inner mitochondrial membrane, where it serves as substrate for P450scc and initiates steroidogenesis (Waterman MR. *Science* 1995;267:1780). StAR is required for steroidogenesis in the adrenal and gonads but not in the placenta. StAR, which is responsive to corticotropin, is a 285 amino acid protein with a 25 amino acid mitochondrial targeting sequence. It is cleaved after entering the mitochondrion.

Figure 1



Putative site of StAR action: Movement of cholesterol from the outer mitochondrial membrane across the intermembrane space to P450scc in the inner membrane. The cholesterol transporter is hypothetical.

In 3 unrelated patients with lipoid adrenal hyperplasia, 2 separate base pair changes were found in the StAR gene: (1) in patient 1, a C → T transition in codon 193 (Arg) resulted in a premature stop codon that is 93 amino acid residues shorter than the mature product; (2) in patients 2 and 3, a C → T transition at codon 258 (Gln) resulted in a premature stop codon and a truncated protein 28 amino acids shorter than the mature protein. Expression of these truncated products in COS-1 cells revealed absent steroidogenesis with cholesterol as substrate. Interestingly, with 20α-hydroxycholesterol as substrate, steroidogenesis was normal in these cells.

Lin D, et al. *Science* 1995;267:1828-1831.

***Editor's comment**: The essential role of the StAR accessory protein in the mobilization and transmitochondrial membrane transport of cholesterol for steroidogenesis is in the adrenal and gonads but not in the placenta, since progesterone production is normal in pregnancies in which fetuses have lipoid adrenal hyperplasia. Since StAR is not expressed in the placenta and brain, another mitochondrial cholesterol transport system must be present in these tissues. The observation that when 20α-hydroxypregnenolone is employed as substrate steroidogenesis is normal in defective cells suggests that this compound may be of therapeutic benefit to patients with lipoid adrenal hyperplasia. Of the several forms of congenital adrenal hyperplasia, lipoid adrenal hyperplasia is the only one that is not due to a molecular defect in a steroidogenic enzyme.*

Allen W. Root, MD

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc. The information in this publication reflects the views of the editors and/or contributors and not necessarily those of the grantor or the publisher.

Published by:
Gardiner-Caldwell
SynerMed
405 Trimmer Road
PO Box 458
Califon, NJ 07830

Evidence for Partial Growth Hormone Insensitivity Among Patients With Idiopathic Short Stature

Reported in this study are 511 children with idiopathic short stature (ISS) (height standard deviation score [SDS] of ≤2; maximum stimulated growth hormone [GH] >10 µg/L; and no other reason for short stature) who were treated with GH. Growth hormone-binding protein (GHBP) was measured before GH treatment. In 101 (20%) patients GHP SDS ≤ -2, whereas in the remaining 410 (80%) patients GHP SDS > -2. Patients with low GHBP levels had lower mean extracted insulin-like growth factor 1 (IGF-1) SDS (-3.3 ± 1.1 vs -2.5 ± 1.4; *P*<0.0001) and higher mean 12-hour GH values (2.8 ± 1.1 vs 2.3 ± 1.1 µg/L; *P*<0.0001) when compared with patients with normal GHBP levels. A direct correlation was found between GHBP SDS and extracted IGF-1 SDS, whereas an inverse correlation was present between GHBP SDS and mean 12-hour GH values. Growth velocity before and after 1 year of treatment with GH was not different between prepubertal patients with low and normal GHBP. No correlation was found between first-year growth rate with GH treatment and GHBP SDS. The authors conclude that ISS patients who have low levels of GHBP are partially insensitive to GH, as suggested by a lower IGF-1 and a higher 12-hour mean GH concentration. The authors also present a proposal for a redefinition of normal growth and growth disorders based on the evaluation of endogenous GH secretion and GH responsiveness assessed by the GHBP.

Attie, KM, et al. B.M. *J Pediatr* 1995;127:244-250.

***Editor's comment**: This is an excellent study with a large number of short-statured patients studied in a sophisticated prospective manner. Unfortunately, the authors did not separate the results by growth velocity measured before and after treatment with GH. They reported a mean pretreatment growth velocity of 4.0 ± 1.7 cm/y in the low GHBP group and of 4.2 ± 1.9 cm/y in the normal GHBP group. The great variability implied by the mean ± 2 SD (ie, growth velocities before initiation of therapy ranging from 0.6 to 7.7 cm/y and from 0.4 to 8.0 cm/y, respectively) indicates that there were some patients who were growing very well and some others who were growing poorly. The responses to GH were also reported as a mean of the whole group, thus individual variations cannot be discerned. Patients growing at a decreased rate who significantly increased their growth after GH treatment would differ from those patients originally growing at normal rates who had a minimal increase of growth rate after GH treatment. These data are important to understand the significance of the findings reported.*

The more we look for magic bullets to diagnose growth abnormalities, the more compelling becomes the old adage: careful measurements of growth velocity are necessary to ascertain the need for therapy and the response to it. The reader is referred to a recent article in the New England Journal of Medicine (1995;333:1093-1098); for a report of the mutations of the GH receptor in children with ISS.

Fima Liftshitz, MD

GROWTH, Genetics, & Hormones is published under an educational grant from Genentech, Inc.

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